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Crustacea

Edited by Genaro Diarte-Plata and Ruth Escamilla-Montes

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Meet the editors

Dr Diarte-Plata is a professor of research with an appointment candidate level in the SNI-CONACYT Mexico. He received his BSc degree in aquaculture at the Universidad Autónoma de Sinaloa, Mexico. He has his Masters degree in natural resources and environment from the Instituto Politécnico Nacional (IPN CIIDIR-Sinaloa), Mexico. He is currently a professor at the Instituto Politécnico Nacional, Mexico (since 2006). He is also a researcher of the Sinaloa System of Researchers and Technologists (SSIT-INAPI Sinaloa). He received his PhD in marine and coastal sciences at the Universidad Autónoma de Baja California Sur, Mexico. His research topics include investigations in marine, culture and ecology of molluscs and crustaceans. As a professor-researcher within the IPN CIIDIR-Sinaloa, he has participated in 48 research projects, given 11 seminars, 260 presentations at international and national conferences, written 5 book chapters, and 19 indexed articles. He teaches descriptive and multivariate statistics at the undergraduate level, and aquaculture at the postgraduate level. He has been the director of 15 undergraduate theses and 5 Masters and 2 Doctorates from various universities. He is the arbitrator in the revision of manuscripts sent to international journals (ISI-JCR).

Dr Escamilla-Montes received her doctorate in marine and coastal sciences with a specialty in aquaculture. She is an assistant technologist of the Sinaloa System of Researchers and Technologists. Her areas of study are cultivation and ecology of molluscs and crustacean. In the last five years, she has focused on the isolation and characterization of lactic acid bacteria, yeasts, and bacilli with probiotic potential and pathogenic bacteria such as *Vibrio parahaemolyticus* and its effect on the growth, survival, and expression of genes of the immune system and stress of marine organisms. She has participated in 11 research projects, as well as presentations at national and international conferences, written 4 books, and 28 articles in ISI Thomson-JCR international journals. She is also the arbitrator in the revision of manuscripts sent to international journals (ISI-JCR).

Contents

Preface

Crustaceans form one of the most successful zoological groups, both for the number of registered living species (67,829) and for the diversity of habitats that they colonize (from the shallow coastline to the deep ocean basins). They are organisms that have various anatomical variations and dimensions ranging from a few millimeters (some copepods) to more than 4 m in length (spider crab). The variety of life strategies has allowed them to adapt to a wide variety of environments, first marine, then freshwater, and finally terrestrial. As for food, there are detritivores, herbivores, omnivores, carnivorous scavengers, and parasites.

The importance of crustaceans not only lies at the food level, but they also provide multiple benefits to man: in the commercial field (through the exploitation of different species, which means significant economic income worldwide); in the pharmaceutical, food, and beverage industry; in water treatment; in agriculture; in medicine (chitosan is used, which is extracted from the main component of the shell that forms the body of organisms, chitin); and in the food and dye industry by the use of carotenoids (astaxanthin) extracted from the lobster exoskeleton (*Pleuroncodes planipes*) to give a reddish color to different foods. In the biological aspect, crustaceans are fundamental in the functioning of aquatic ecosystems; with their abundance and diversity they give structure to the macroinvertebrate communities. Because of the position they occupy in the food chains, as secondary consumers, they are responsible for the transformation of organic matter into energy and consumable proteins.

This book is made up of five sections. The first section is about **Biology and Ecology.** The first chapter is ***Crustace***an, where aspects of all the animals of the phylum Arthropoda and Subphylum Crustacea are discussed. This group of animals are a very diverse group of invertebrate animals, which includes active animals such as crabs, lobsters, shrimp, krill, copepods, amphipods, and more sessile creatures like barnacles. It includes about 11,340,000 species in all habitats. This constitutes about 83% of all the known animal species on earth. The phylum is characterized by heteronomous metamerism, chitinous exoskeleton, and joined appendages. In very small crustaceans, the exchange of respiratory gases occurs through the general body surface. Large aquatic arthropods respire through gills and book gills, whereas terrestrial forms respire through trachea and book lungs.

The chapter entitled ***The robber crab Birgus latro (Linnaeus, 1767)*** gives us information on the terrestrial hermit crab. It is the largest land-living arthropod in the world. In India, distribution of this crab is restricted to Great Nicobar Island and South Sentinel Island in Andaman and Nicobar Archipelago. This research is focused on aspects of ecology such as habitat, size, eating habits, behavior, life cycle, and distribution on the Nicobar Islands, India.

In the chapter ***Scyllarid Lobster Biology and Ecology,*** the current available knowledge on the biology of scyllarids is presented. The author attempts to point out where questions remain to help focus further studies on these lobsters, while also mentioning that slipper lobsters are found in tropical and temperate habitats with hard or soft undercarriages and at different depths, and exhibit a wide array

of morphological, anatomical, and physiological adaptations. Amongst the 20 genera and at least 89 species constituting four subfamilies, only some members of four genera, *Thenus* (Theninae), *Scyllarides* (Arctidinae), *Ibacus,* and *Parribacus* (Ibacinae) form significant fishery crops because of their large size.

To the chapter titled ***Management of the interaction and cannibalism of postlarvae and adults of the freshwater shrimp Cryphiops caementarius (Molina, 1782),*** mentions that male shrimps (*C. caementarius)* are aggressive because one of their chelipeds is more developed than the other, causing greater interaction and cannibalism in any aquaculture system. Female shrimps are less aggressive. To reduce interaction and shrimp cannibalism, two management systems are proposed. For postlarvae, using brackish water (12%) maintains high survival (> 85%) but only in the initial culture, which lasts 50 days. For the fattening of adult males, growing in individual containers conditioned in multiple levels, allows high survival (87% and 100%) and yields between 0.7 kg m^{-2} and 1.0 kg m^{-2}. Furthermore, in this system, the co-culture shrimp/tilapia is also tested to maximize performance. Study is still required to demonstrate the technical and economic feasibility of fattening male shrimps in individual containers within semi-natural ponds.

In the chapter ***Bateman Gradients and Alternative Mating Strategies in a Marine Isopod,*** the Bateman gradient is shown to provide a means for estimating the strength of sexual selection. By measuring the covariance between mate numbers and offspring numbers for members of each sex, this parameter identifies the existence and magnitude of sex differences in selection intensity. Although widely used for this purpose, this approach has not been applied to examine the covariance between mate numbers and offspring numbers among alternative mating strategies. Differences in this covariance could exist if the average fitness of different mating phenotypes was unequal, as has been suggested for alternative mating tactics.

The chapter ***The habitat types of freshwater prawns (Palaemonidae: Macrobrachium) with abbreviated larval development in Mesoamerica (Mexico, Guatemala & Belize)*** describes the freshwater prawns of genus *Macrobrachium* with abbreviated larval development that have been reported from South America, Asia, Africa, and North America. They have been collected from a diversity of freshwater habitats (caves, springs, and primary streams from so-long basins). This chapter details the diversity of habitats of these freshwater prawns that show a high plasticity in their morphological features associated with these different habitats.

In the second section, **Fisheries,** the first chapter, ***Estimation of the maximum sustainable yield and the optimal fishing effort of the blue crab (Callinectes sapidus, Rathbun 1896) of Laguna Madre, Tamaulipas, Mexico,*** describes the economic and social importance of the crab fishery industry from Mexico, and in particular the blue crab (*Callinectes sapidus*) on the coast of the Gulf of Mexico, specifically in the Laguna Madre, Tamaulipas. The official measures of fishing management are insufficient and outdated; while those corresponding to unofficial scientists are nonexistent. On the Pacific Ocean side, several species of the genus *Callinectes* concur for which certain regulations (Official Mexican Standard) and planning (Regional Fisheries Management Plan) applicable to fisheries regulation are available. In the specific case of Laguna Madre, Tamaulipas, there is currently no specific regulation or regional fisheries planning.

The third section, **Genetics,** includes the chapter ***A Comparison of Genetic Variation in Two Endemic Thermal Spring Isopods, Thermosphaeroma***

thermophilum and T. milleri (Crustacea: Isopoda: Sphaeromatidae), where *Thermosphaeroma thermophilum*, an endangered species, inhabits a single thermal spring in central New Mexico, USA. *T. milleri* inhabits a more complex thermal spring system in northern Chihuahua, Mexico. We found no significant differences in allelic variation between the sexes within each species. Between species, electromorphs at each locus differed significantly in both number and moiety on the gel, with *T. milleri* showing greater polymorphism and greater heterozygosity than *T. thermophilum* suggesting that these populations have been separated since the late Cretaceous period (88 myr). Moreover, consistent with the theoretical expectation that small, isolated populations will exhibit reduced genetic variation, *T. thermophilum* exhibits significantly less genetic variation than the more numerous and less confined *T. milleri*.

In the fourth section, **Diseases,** a chapter on ***Phage therapy for control of bacterial diseases of crustaceans*** describes one of the most important control strategies envisaged for the management of bacterial diseases in the aquatic environment. There are no other effective alternative approaches for the natural control of bacterial diseases and phage therapy remains the best method that has not yet been exploited. The occurrence, infectivity, lytic activities, therapeutic potentials, and efficacy of the bacteriophages of *Bacillus spp.*/Vibrio *spp.* for control of pathogenic bacteria diseases such as *Vibrio vulnificus, V. damsela,* and *V. furnissi* in the cultures of crustaceans are presented. An ideal method for long-term storage and recovery of the lytic bacteriophages and validation of the usefulness of phage therapy are reviewed. The application and efficacy of the phages of *Bacillus/Vibrio* against the bacterial pathogens *Vibrios* in the aquaculture of crustaceans are considered.

The fifth section, ***Bioaccumulation,*** provides information on ***Assessment of metal accumulation and bioaccumulation factor of some trace and heavy metals in freshwater prawn and crab,*** describing the freshwater decapods that are a major food delicacy because of their rich deposits of minerals. High levels of mineral contents such as metals are usually accumulated in the body tissues of these organisms because of their lifestyle. Metal accumulation in freshwater decapods has been thought to cause serious health concerns when transferred to humans along the food chain. Health risks associated with heavy metals include renal failure, skeletal deformation, and hepatic failure. The assessment of metal accumulation and bioaccumulation factors of some trace and heavy metals in freshwater prawns and crabs (*Sudanonautes africanus* and *Macrobrachium rosenbergii*) have shown that the metal accumulation were in this order: Fe > Zn > Cu > Pb = Cd > Cr = Ni = V and Fe > Zn > Mn > Cu > Pb > Cr = Cd > Ni = V. It was noticed that Zn and Cr had the highest bioaccumulation factors in prawns and crabs respectively. Chromium has been observed to be carcinogenic. Consumption of Cr in the muscles of crab might cause serious health risks to humans.

This book can be used by students, professors, and researchers in areas related to biological sciences.

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Section 1

Biology and Ecology

Chapter 1

Crustacean

Imad Mahmood Ghafor

Abstract

Crustaceans include all the animals of the phylum Arthropoda Crustacea; the word comes from the Latin *crusta*, which means shell. Crustaceans are a very diverse group of invertebrate animals which includes active animals such as the crabs, lobsters, shrimp, krill, copepods, amphipods, and more sessile creatures like barnacles. Arthropoda is the largest phylum of Animal Kingdom. It includes about 11,340,000 species in all habitats. This constitutes about 83% of all the known animal species on earth. Arthropoda includes spider, scorpions, prawns, crabs, millipedes, centipedes, and many other insects. Arthropoda is characterized by heteronomous metamerism, chitinous exoskeleton, and joined appendages. The evolutionary acquisition of these traits is known as arthropodization. In very small crustaceans, exchange of the respiratory gases occurs through the general body surface. Large aquatic arthropods respire through gills and book gills, whereas terrestrial forms respire through trachea and book lungs.

Keywords: Arthropoda, exoskeleton, crustacean: burrow, sedimentology, Ostracoda

1. Introduction

Crustaceans are cladocerans if they have 4–6 pairs of (thoracic) legs, lack any paired eyes, swim with their second pair of antennae, and have at least the head not covered by a carapace [1]. Crustaceans are some of the most important marine life to humans—crabs, lobsters, and shrimp are widely fished and consumed around the world.

There are more than 52,000 species of crustaceans in the world, which include popular marine animals like lobsters, crabs, shrimp, crayfish, and barnacles. Smaller crustaceans breathe through their bodies and larger ones breathe through gills. Most crustaceans are dioecious, meaning individuals are male or female. Reproduction varies among species. Most of them are the most important marine animals. Humans rely heavily on crustaceans for food; and crustaceans are also an important prey source for marine life in the ocean food chain for a variety of animals, including whales, fish, and pinnipeds; more diverse than any group of arthropods, crustaceans are second or third in abundance of all categories of animal life after insects and vertebrates. They live in inland and ocean waters from the Arctic to the Antarctic as well as from elevations in the Himalayas up to 16,000 feet to well below the sea level. All crustaceans have a hard exoskeleton which protects the animal from predators and prevents water loss. However, exoskeletons do not grow as the animal inside them grows, so crustaceans are forced to molt as they grow larger. The molting process takes between a few minutes to several hours. During molting, a soft exoskeleton forms underneath the old one and the old exoskeleton is shed. Since the new exoskeleton is soft, this is a vulnerable time for the crustacean until

the new exoskeleton hardens. After molting, crustaceans typically expand their bodies almost immediately, increasing by 40–80%. Most crustaceans reproduce sexually with a separate male and female.

2. General characters of phylum Arthropoda

Arthropoda have the following description [2]:

1. Cosmopolitan in distribution is found in aquatic, terrestrial, and aerial forms.
2. Body has jointed appendages or legs.
3. Body is triploblastic.
4. Bilaterally symmetrical.
5. Organ system level of organization.
6. Body is divisible into head, thorax and abdomen.
7. Segmented.
8. Jointed appendages.
9. Hard external skeleton.
10. Three parts (head, thorax, and abdomen).
11. Exoskeleton composed of chitineous materials.
12. Growth type is by molting which sheds old skeleton and secretes a large one.
13. They are either oviparous or ovoviviparous.

3. Classification of phylum Arthropoda

Phylum Arthropoda have different views concerning their phylogeny. So there is no absolute system of classification for this phylum. The below given classification is the most accepted one. Arthropoda classified into subphylums and classes three subphyla namely Trilobita, Chelicerata, and Mandibulata are definitively arthropods, classes Trilobita, Xiphosura, Arachinida, Crustacea, Cheliopoda, Diplopoda, and Hexapoda [3] (**Table 1**).

4. Classification of the crustaceans

Crustaceans have been known to humans since ancient times and have provided us with sources of both food and legend. The classification of crustaceans has been quite variable; the system used by [4] presented an overview of crustacean classification, and readers are referred to that publication for a window into the labyrinthine history of this subphylum. This classification was recognized in [5, 6] (**Table 2**).

Phylum	**Arthropoda**		
Subphylum	Trilobata Ex. *Trithurus*	Chelicerata	Mandibulata
Class		Xiphosura Ex. *Limulus*	Crustacea Ex. *Palaemon*
		Arachinida Ex. *Palamnaeous*	Cheliopoda Ex. *Scolopendra*
		—	Diplopoda *Ex. Spirobolus*
		—	Hexapoda Ex. *Musca*

Table 1.
Classification of phylum Arthropoda.

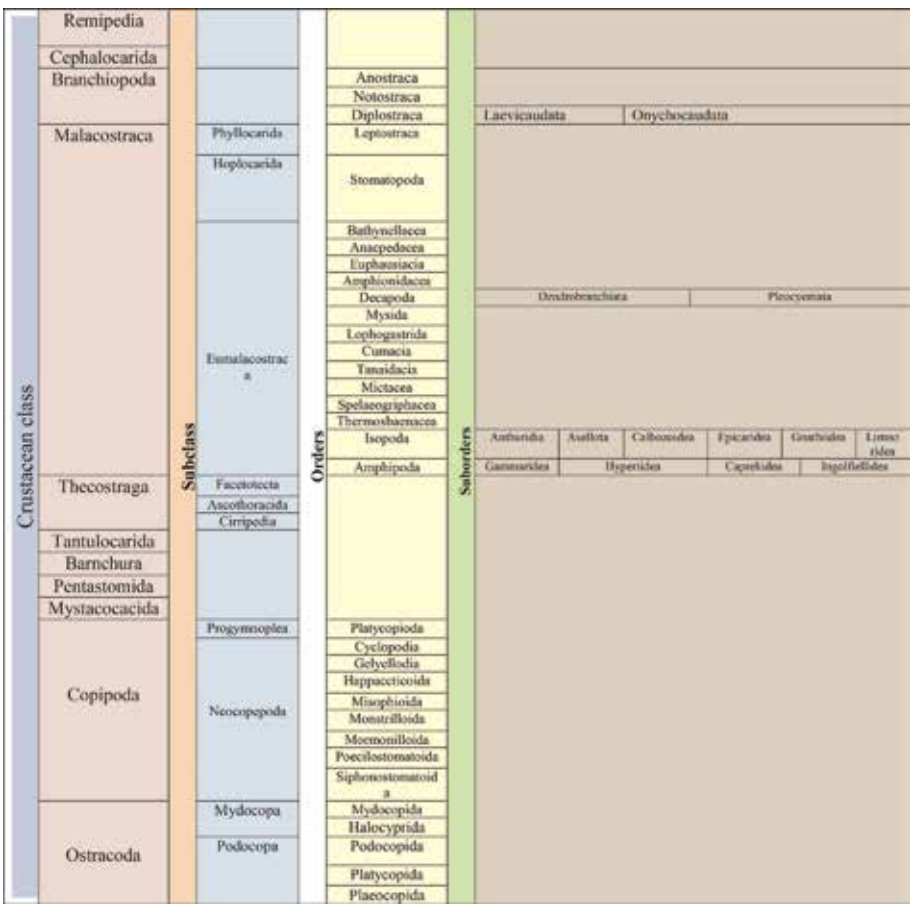

Table 2.
Classification of the Crustacea [25].

5. General characters of class Crustacea (Crusta = shell)

1. Class Crustacea head, or cephalon (plus the acron), thorax and abjedium (**Figure 2**).

2. They are found in marine, fresh water and terrestrial habitats.

3. Possess jaw, like appendages called mandibles.

4. Crustaceans have two pairs of antennae and two pair of maxillae.

5. Some of appendages are biramous.

6. Origin and application and crustacean

Crustaceans have important economic, ecological, and esthetic values and also can be appreciated from the perspective of bi-level functionality. Some larger crustaceans, including shrimp, lobsters, and crabs, are a major food commodity, while smaller crustaceans in their own way are integral to many food webs, sometimes considered a class or superclass rather than a subphylum. The scientific study of crustaceans is known as *carcinology*. Other names for carcinology are malacostracology, crustaceology, and crustalogy, and a scientist who works in carcinology is a carcinologist, crustaceologist, or crustalogist. The origin crustacean differs according to the order, suborder, or other taxons of the crustacean [7]. The earliest crustaceans are known from Cambrian sediments including the well-known Burgess Shale fauna. These primitive crustaceans are essentially worm-like in shape, but they do have many of the key features of crustaceans visible even on modern types such as shrimps [8]. So, the origin is based on the age of the genera and species. The small planktonic and free-swilling crustaceans were common in the Paleozoic era. It is relatively rare to find their skeletons entirely except in those places, like the Burgess Shale, where some catastrophic events smothered them quickly enough trevent their decay [9]. The crustaceans colonized mud firm grounds, which were formed by erosion during a rapid sea-level fall; thus, the burrows occur in direct association with erosional regressive surfaces and therefore are good stratigraphic indicators of abrupt paleoenvironmental change.

7. Ecology

The ecology of the crustacean differs from one type to another. They live in aquatic and terrestrial environments, and all are marine but a few groups have adapted to life on land, such as terrestrial crabs and terrestrial hermit crabs. They are also found as burrowed in the sand of beaches will near access of water. Some freshwater crustaceans are crawfish and fairy shrimp. Crawfish live in lakes and rivers hidden under rocks and sand. Fairy shrimp are found in vernal ponds which are temporary puddles made by rain water. Various species have occupied almost every conceivable niche within the aquatic environment. An enormous abundance of free-swimming (planktonic) species occupies the open waters of lakes and oceans. Other species live at the bottom of the sea, where they may crawl over the sediment or burrow into it. Different species are found in rocky, sandy, and muddy areas. Some species are so small that they live in the spaces between sand grains. Others tunnel in the fronds of seaweeds or into man-made wooden structures. Some members of the orders Isopoda and Amphipoda extend down to the greatest depths in the sea and have been found in oceanic trenches at depths of up to 10,000 m. Crustaceans colonize lakes and rivers throughout the world, even high mountain lakes at altitudes of 5000 m. They range widely in latitude as well: in the high Arctic, some crustaceans use the short summer to develop quickly through a generation, leaving dormant stages to overwinter [10].

8. Life cycle

The life cycle for different crustaceans may be different or they are similarities between one crustacean and the next when it comes to their lifecycles. The Crustacean class is the largest group of arthropods of a marine nature, and there are approximately 30,000 different species in this group alone. The life cycle for different crustaceans is going to have unique qualities, but there are also similarities between one crustacean and the next when it comes to their life cycles.

Nauplius stage—this stage of crustacean life cycle is perceived as being a defining link among all crustaceans. This is the first larval stage of crustaceans and consists only of crustacean head and telson as neither the abdomen nor the thorax has developed [11].

Zoea larval stage—the crustacean life cycle involves a larval stage that is known as a zoea. When the zoea name was given to the crustacean, naturalists believed that it was an entirely separate species.

Mysis or megalopa stage—the stage of growth following the zoea stage of growth is either the Mysis or megalopa stage development on what crustacean group is involved.

The crustacean is going will being to look more like to its adult form. This is also the stage of growth where the crustacean will depend more on foraging and grazing to feed.

Adult growth stage—the adult growth stage is reached by 1 year of age for the most crustacean. After a year has passed, most crustacean varieties will be capable of mating and reproducing [12].

8.1 Mating system

Crustacean produced by sexually: a small number is hermaphrodites, including Barnacles, Remipedes, and Cephalocarida. Some may even change sex during the course of their life. Parthenogenesis is also widespread among crustaceans, where viable eggs are produced by a female without needing fertilization by a male. This occurs in many branchiopods, some ostracods, some isopods, and certain “higher” crustaceans, such as the *Marmorkrebs* crayfish [13].

8.2 Eggs

The fertilized eggs are simply released into the water column, while others have developed a number of mechanisms for holding on to the eggs until they are ready to hatch. Most decapods carry the eggs attached to the pleopods, while peracarids, notostracans, anostracans, and many isopods form a brood pouch from the carapace and thoracic limbs. Female Branchiura do not carry eggs in external ovisacs but attach them in rows to rocks and other objects. Most leptostracans and krill carry the eggs between their thoracic limbs; some copepods carry their eggs in special thin-walled sacs, while others have them attached together in long, tangled strings [14] (**Figures 1** and **2**).

8.3 Larvae

The visual systems of crustacean larvae concentrate on the compound eyes of decapod and stomatopod larvae as well as the functional and behavioral aspects of their vision. Larval compound eyes of these macrurans are all built on fundamentally the same optical plan, the transparent apposition eye, which is eminently suitable for modification into the abundantly diverse optical systems of the adults. Many of these

Figure 1.
Eggs of Potamon fluviatile*, a freshwater crab.*

Figure 2.
Zoea larva of the European lobster, Homarus gammarus.

eyes contain a layer of reflective structures overlying the retina that produces a counter illuminating eye shine, so they are unique in being camouflaged both by their transparency and by their reflection of light spectrally similar to background light to conceal the opaque retina. Besides the pair of compound eyes, at least some crustacean larvae have a non-imaging photoreceptor system based on a naupliar eye and possibly other frontal eyes. Larval compound eye photoreceptors send axons to a large and well-developed optic lobe consisting of a series of neuropils that are similar to those of adult crustaceans and insects, implying sophisticated analysis of visual stimuli. The visual system fosters a number of advanced and flexible behaviors that permit crustacean larvae to survive extended periods in the plankton and allow them to reach acceptable adult habitats, within which to metamorphose [15].

9. Crustacean burrow

Crustaceans are mainly males, excavate burrows largely in carbonate substrates, and are therefore referred to as the burrowing barnacles. While their greatest diversity is found in shallow tropical seas, the most generalized or primitive members are found for the most part in deep water (between 1000 and 3000 m). Trace fossils, ranging back to the Devonian if not the Ordovician [16], reveal that species once occupied relatively high latitudes in Northern Europe and Gondwanaland, and at least one extant species is known from Antarctic waters today.

Interpretation of the crustacean burrows from Mallorca makes them very comparable to some modern and fossil thalassinidean burrow systems [17, 18], and it is a direct consequence of the versatile behavior of fossorial shrimps. The helical burrows described herein were very likely part of complex burrow systems produced by thalassinideans. From an ichnotaxonomic point of view, these would be

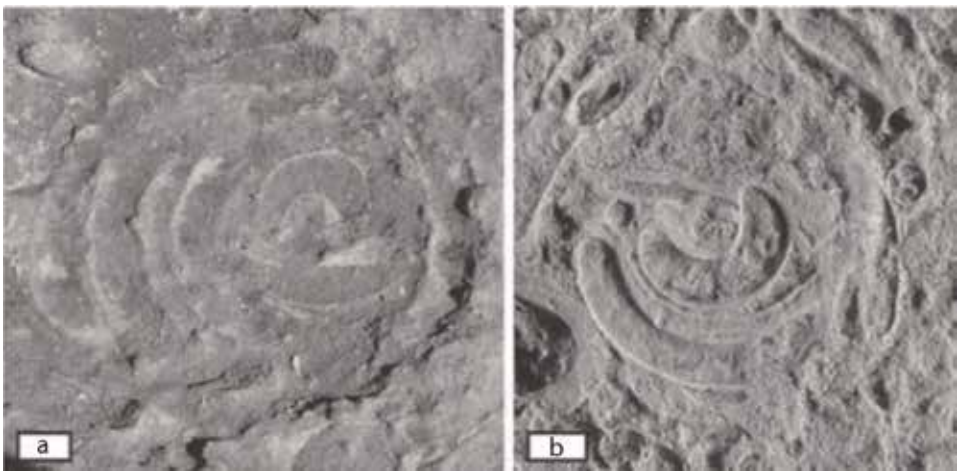

Figure 3.
Helical burrows. (a) and (b) Different parallel-to-bedding sections showing their architectural variability.

Figure 4.
Chthamalus stellatus *(Sessilia).*

Figure 5.
Cylindroleberididae.

compound structures composed of pellet-lined (Ophiomorpha) and unlined (Thalassinoides) branching tunnels, sometimes with spreiten due to vertical shifting (Teichichnus), or double (Lapispira) helical elements. Such double helix elements (Lapispira) were previously known only from the Jurassic as isolated burrows, also assigned to crustaceans [13]. Despite the lesser geometric regularity of the Mallorcan burrows, the presence of a knobby lining and the fact that these may

Figure 6.
Fossil remains of a barnacle (Cirripedia—left) and a crab (Decapoda—right) found in the UCMP teaching collection (images by Karen Osborn). Fossil stomatopod, center (image by Dr. Cees Hof, used with permission).

Figure 7.
Crayfish.

Figure 8.
Etyus martini.

Figure 9.
Spiny lobsters.

be connected to branching systems. The new occurrence of this unusual ichnogenus may record a case of behavioral convergence expressed in burrow architecture [19] (**Figures 3–6**). While most crustaceans are marine, a large number of crayfish live in freshwater, including crayfish (**Figure 7**). *Etyus martini* is one of the more common crabs in the Gault Clay (**Figure 8**). Spiny lobsters are among the larger crustaceans. Big specimens can weigh several kilograms and make very good eating (**Figure 9**).

10. Geological history

The crustaceans, such as crabs and lobsters, that have hard exoskeletons reinforced with calcium carbonate tend to preserve well as fossils, but many crustaceans have only thin exoskeletons. Most of the crustacean fossils known are from coral reef or shallow sea-floor environments, but many crustaceans live in open seas, on deep sea floors, or in burrows. Crustaceans tend, therefore, to be rare in the fossil record than trilobites. Some crustaceans are reasonably common in Cretaceous and Cenozoic rocks, but barnacles have a particularly poor fossil record, with very few specimens from before the Mesozoic era. The Late Jurassic lithographic limestones of Solnhofen, Bavaria, which are famous as the home of *Archaeopteryx,* are relatively rich in decapod crustaceans (five pairs of legs), such as *Eryon* (an eryonoid), *Aeger* (a prawn), or *Pseudastacus* (a lobster). The "lobster bed" of the Greensand formation from the Cretaceous period, which occurs at Atherfield on the Isle of Wight, contains many well preserved examples of the small glypheoid lobster *Mecochirus magna*. Crabs have been found at a number of sites, such as the Cretacoues Gault clay and the Eocene London clay.

11. Crustacean example: ostracods

Ostracods are tiny crustaceans, typically about one to two millimeters in length, with a well-documented fossil record beginning in the early Ordovician (e.g., [20–24]). During the Ordovician period, ostracods already possessed a global

biogeographical distribution from high southern latitudes to the palaeo-tropics [26]. Crustacean ostracods are variously represented in washing residue and thin sections, two valves (left and right valves), the two valves being joined together along the hinge line. The body covered by external shell called Carapace is composed of two valves connected in the Dorsal side. Two valves are equal in the genus ***Amphisites*** or overlapping in *Cytherella.* Ovoid shape or semi ovoid, 0.5–4 mmlength to about 30 mm. Articulation along the dorsal margin is further characterized by development of teeth, socket, ridges, and grooves all together called hinge element (hinge elements: teeth, socket, grooves, and ridge-bar). The body is subdivided into Cephalon, Thorax, and Posterior, seven pairs appendages (antenna, antennule, mandible, maxilla, 1st thoracic leg, 2nd thoracic leg, and 3rd thoracic leg), one eye center, and two lateral calcareous part—internal and outer lamella with the valves are hard calcareous part, Carapace—right and left valve connected with hinge (**Figure 10**).

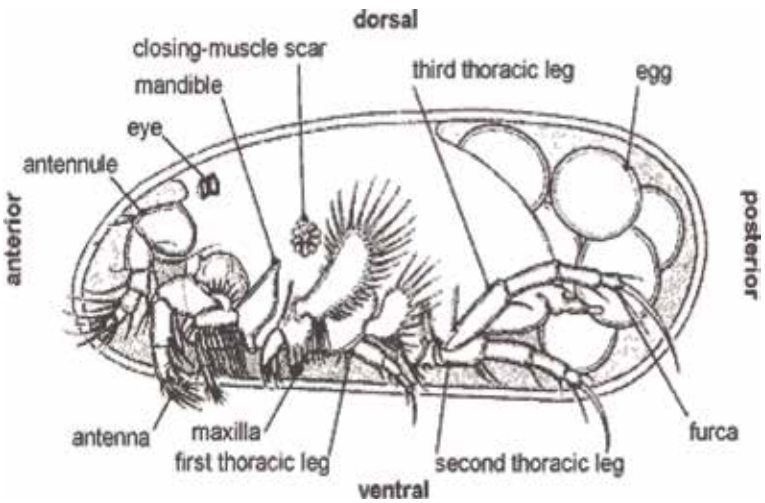

Figure 10.
Ostracoda shell.

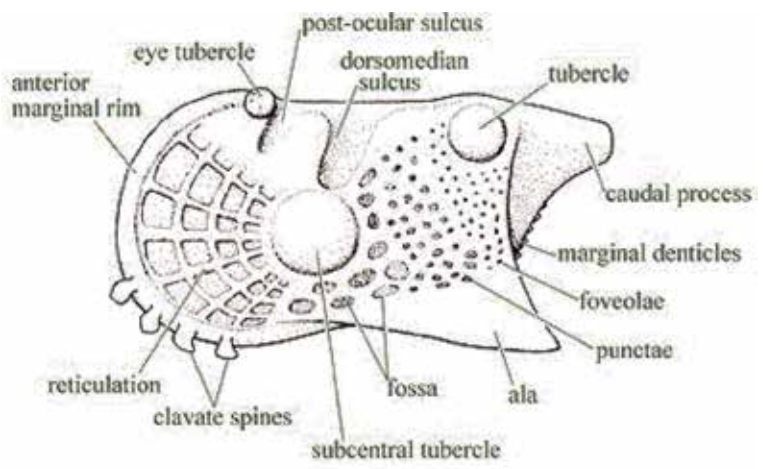

Figure 11.
Ornamentation in some species of Ostracoda.

11.1 Important part in the general shape of ostracods, on the external surface of the test

1. Marginal denticulation: most of the species in Ostracoda have more denticulations (resemble to tooth) accumulated on the external margin of the valves; the number and shape of these denticulation differ from one sp. to another sp. and these denticulation are more accumulated on the anteroventral and posteroventral of the test.

2. Caudal process: some of the species in Ostracoda are characterized by having elongated end that is long and narrow and ended by anus. This caudal process is on the mid-posterior or on the posterodorsal side or posteroventral side.

3. Hinge ears: some species of Ostracoda have protuberance on anterior side of the hinge line which is formed by addition of calcareous materials.

4. Posteroventral spine: it is an calcareous spine on the posteroventral side, usually to the posterior side.

5. Eye tubercle: it is a protuberance on the anterior side which is the position of eye.

6. Anteroventral beak-rostrum (*Cypridea*): some genera of Ostracoda are characterized by having protuberance resembling to beak, which is most abundant in the genus *Cypridea*.

11.2 Ornamentation

Is shown in the carapace view. The outer surfaces of the ostracod valves can be smooth or ornamented with pits, striations, reticulations, spines, sulci, tubercles, and wing-shaped (alae) (**Figure 11**) [22].

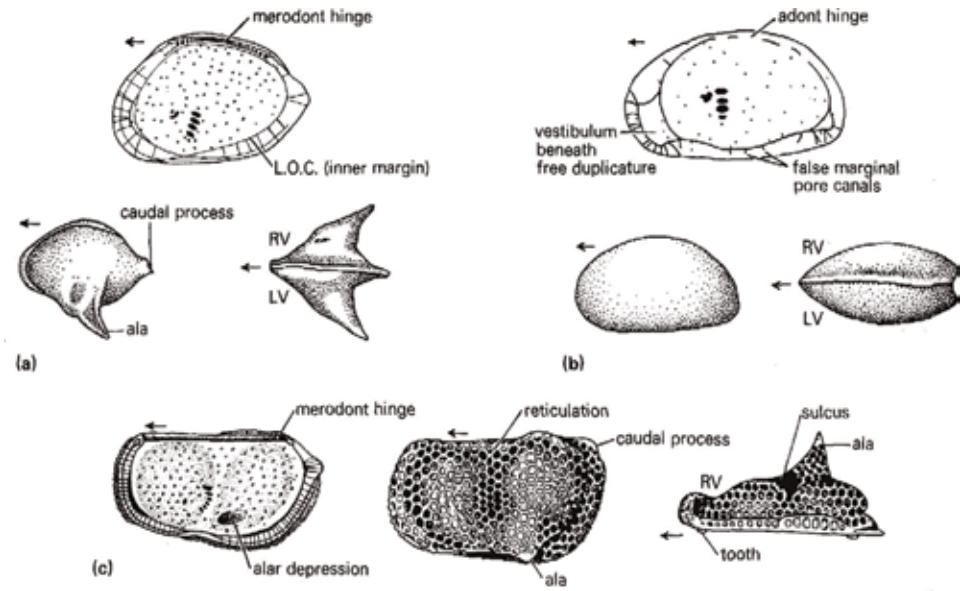

Figure 12.
Important parts in Ostracoda. (a) Caudal process and alae structure (b) Side view showing right and left valves (c) Merodont hinge and alae structure, reticulation.

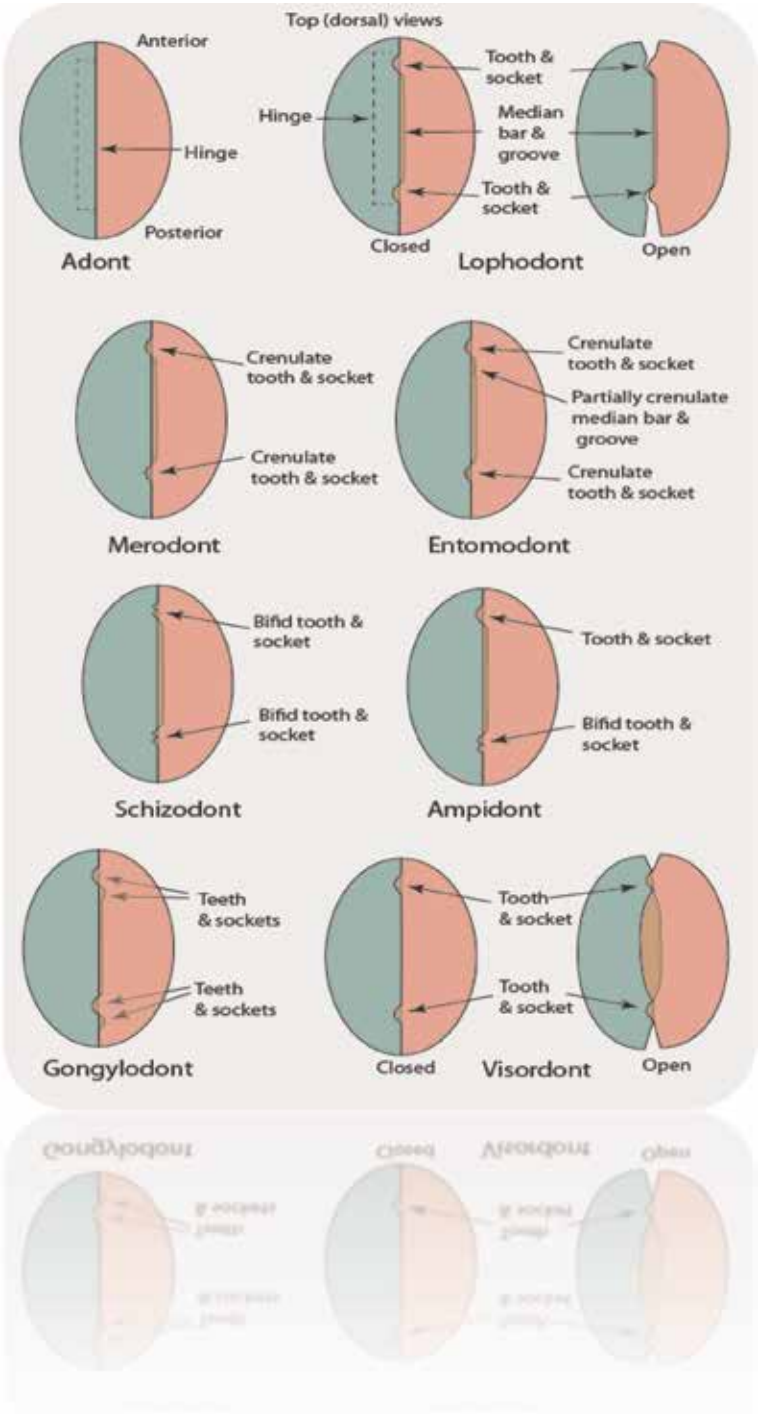

Figure 13.
Teeth types in Ostracoda.

11.3 Pores in ostracods

Normal pores (open normal pores and sieve normal pores) and open normal pores: these pores penetrated the carapace, but sieve normal pores penetrated the

wall. Marginal pores: these pores penetrated the test wall vertically and are distributed on the external surface; the number of the pores differ from family, genus species, and important in classification. Marginal pore canal: long pores distributed on the marginal zone; more pores on the anterior part than the other parts are also important in the classification**.** Test in ostracods is composed of calcareous material with chitineous test around them which helps to fix the hinge. Hinge elements are (Hinge elements: teeth, socket, grooves, ridge-bar) (**Figure 12**).

11.4 Teeth

The teeth in crustacean differ from one taxon to another; for example, ostracods have Adont hinge which is the simplest, without teeth or sockets, and often form part of a contact groove on the larger valve and a corresponding ridge on the smaller valve. The Merodont hinge is composed of a tooth and socket at each end of a groove or ridge structure (complementary negative and positive structures in left and right valves). The Entomodont hinge differs from the merodont hinge style by having a coarsely crenulated anterior portion of the median groove/ridge element. The Amphidont hinge has a more complex median structure with an anterior tooth and socket (**Figure 13**).

11.5 Distribution and ecology of ostracods

Ostracods as a mode of life are pelagic (planktonic) by using organic-walled shell (less $CaCO_3$) or by producing oil droplets. Pelagic ostracods are not preserved in the sediments, or benthic on/in the sea floor. They can burrow, swim near the sea-bed, or crawl on or through the sediment. Benthic forms occur in all the aquatic environments from the abyss to the shoreline. They also occur in estuaries, lagoons, freshwater lakes, ponds and streams, salt lakes, hot springs, and damp vegetation (**Figure 14**).

Ostracods can be influenced ecologically by various factors such as [27]:

1. Type of the substrate: swimmers have smooth, thin, bean-shaped carapace; fine-grained (mud) dwellers have flattened ventral, wing-shaped carapace;

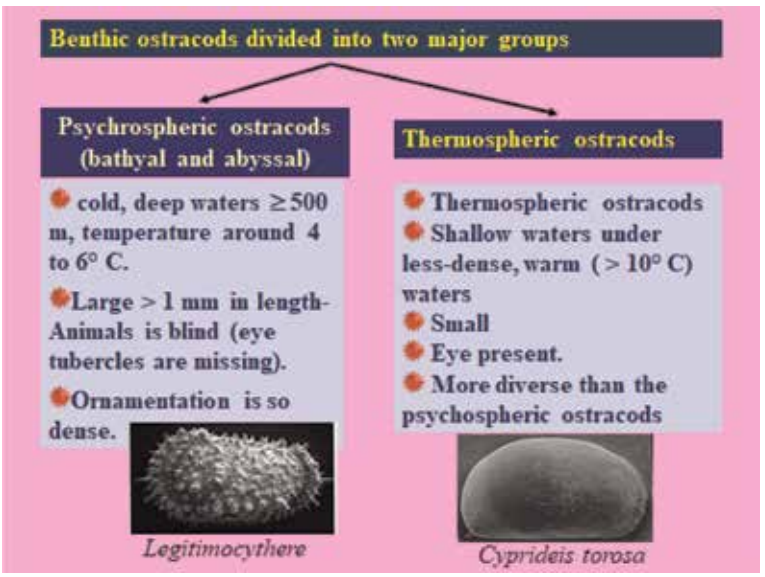

Figure 14.
Psychrospheric and thermospheric ostracods.

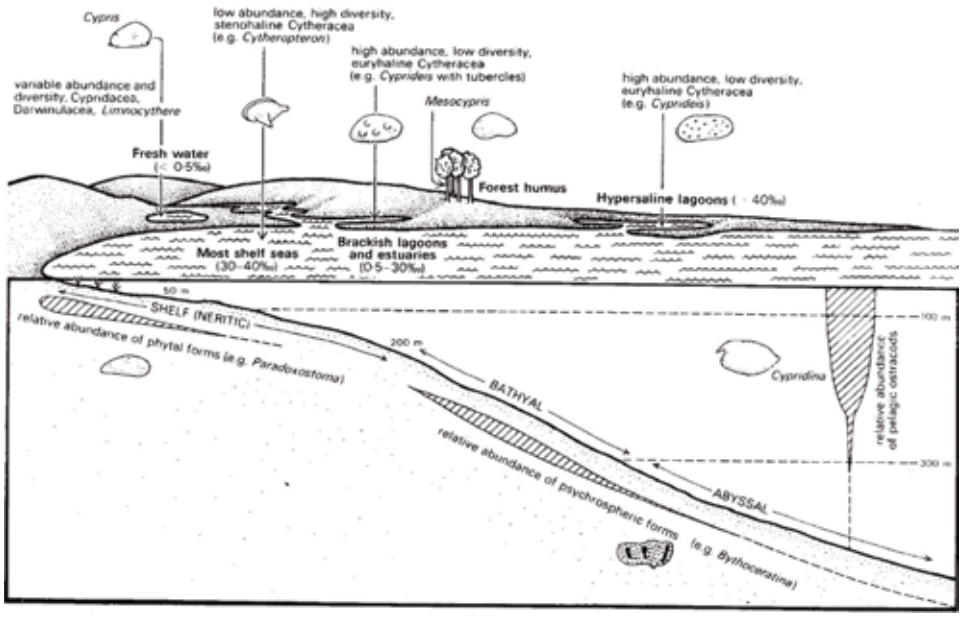

Figure 15.
The ecological distribution of recent Ostarcoda with some tropical represented, Brassier. 2004.

coarse-grained (sand) dwellers have thick carapace with coarse ornamentation; and interstitial ostracods are small, long, and robust.

2. Salinity: ostracods carapace morphology tends to vary according to variation in salinity**.** They occur in fresh water (0.0–0.5‰) of rivers and estuaries, brackish water (0.5–30‰) of lagoons and marshes, normal sea water (35–45‰), and hypersaline water bodies (up to 57‰) of the closed seas, lakes, lagoons, and marginal bays.

Fresh water ostracods—simple morphology, hinge adont, thin carapace, no marginal pores, and other weakly developed variable abundance and diversity.

Most shelf seas ostracods: low abundance, high diversity, stenohaline *Cytheropteron.*

Brackish lagoon and estuaries ostracods: thick shell, weakly ornamented, marginal pore canal, amphidont hinge. High abundance, low diversity, euryhaline, *Cyprideis* with tubecles.

Hypersaline lagoons ostracods, high abundance, low diversity, euryhaline, *Cyprideis.*

Marine ostracods-continental shelf: strongly calcified carapace, strongly ornamented, hinge well developed (**Figure 15**).

11.6 Application

They occur in the sedimentary column since the early Ordovician; hence, they can be used as: stratigraphic markers, paleo-salinity indicators, paleo-depth indicators, biostratigraphy, biostratigraphic correlation, and in paleoecology.

They are used as:

1. Tools for biozonation of marine strata, as they occur from Cambrian to the present.

2. Indicators of ancient marine shorelines salinity, relative sea-floor depth.

Ostracods are used for ecostratigraphy. Ecostratigraphy is the study of the occurrence and development of fossil communities throughout geologic time, as evidenced by biofacies, with particular reference to its relevance in stratigraphic correlation and other fields, such as biogeography and basin analysis. Ecostratigraphic studies by ostracods are based on their morphological changes and ornamentations, which are divided into different biozones and as environmental zone based on, diversity, community, and species abundant, range of the species and environment.

11.7 Ostracods and sedimentology

The genera *Karsteneis karsteni* and *Cythereis longaeva* shows that the ratio of closed valves that the ratio of closed valves (carapace) of high percent and thick in the center of the cretaceous basin (rapid rate of deposition) in bohemia than the other deposits along the sides which are thin sediment and of low rate of deposition.

Example no. 1. Some ostracods in the Garagu Formation, Dhouck City, Kurdistan Region, North Iraq (**Figure 16**) [28].

Example no. 2. The biostratigraphic distribution of Late Ordovician ostracod faunas from the Ellis Bay Formation on western Anticosti Island are described. Some 62 species are recorded. The EllisBay Formation can be subdivided into three ostracod biozones (these being partial range zones) and an interregnum, in ascending stratigraphical order these being the *Longiscula subcylindrica* biozone, the *Eurychilina erugoface* biozone, the *Tetradella anticostiensis* biozone and an interregnum in the uppermost part of the succession, marked by the local extinction of several taxa at the terminus of the *T. anticostiensis* biozone. These intervals are only locally developed, and are not useful for inter-regional correlation. A small number of the Ellis Bay Formation ostracod species are recorded elsewhere, from Sandbian and Katian age successions. These include *Aechmina richmondensis*, *Aechmina maccormicki*, *Baltonotella parsispinosa*, *Macrocyproides trentonensis*, *Microcheilinella lubrica* and *Spinigerites unicornis* [29].

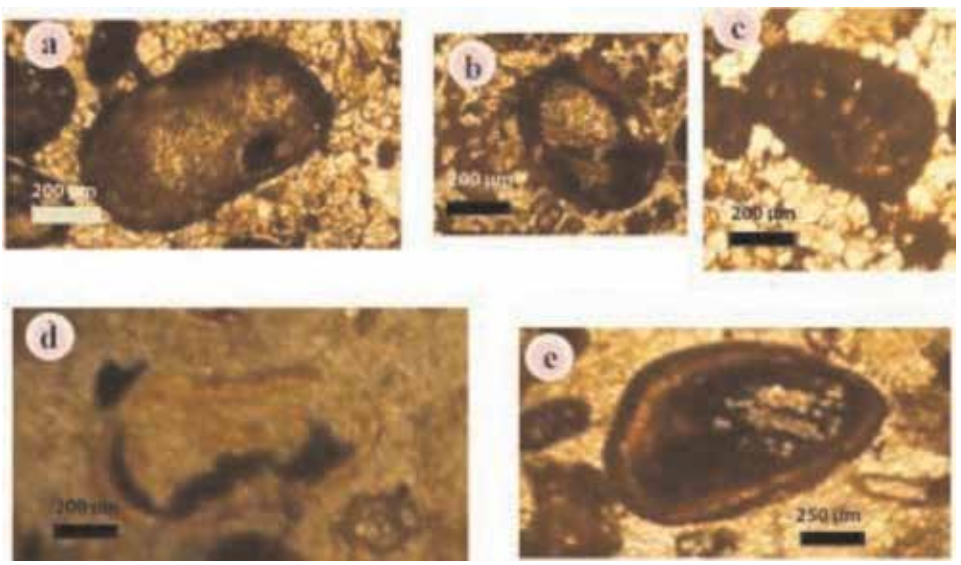

Figure 16.
(a) Cytherella *sp., (b)* Neocythere cf. gottisi *Damotte & Grosdidier, 1963, (c)* Protocythere bedoulensis *Moullade 1966, (d)* Rehacythereis bernardi, *(e)* Cypridea bispinosa *(Jones 1878) [28].*

12. Conclusions

This chapter has the following conclusions:

Crustaceans (Arthropods) are a group of animals with an armored external skeleton (called an exoskeleton),

1. The hard exoskeleton is the part that is preserved as a fossil. Arthropod comes from the Greek words "arthro" meaning joint and "poda" meaning foot or leg.

2. Arthropoda is the largest phylum of Animal Kingdom. It includes about 11,340,000 species in all habitats.

3. Arthropoda is characterized by heteronomous metamerism, chitinous exoskeleton, and joined appendages.

4. In very small crustaceans, exchange of the respiratory gases occurs through the general body surface.

5. Large aquatic arthropods respire through gills and book gills, whereas terrestrial forms respire through trachea and book lungs.

6. The earliest crustaceans are known from Cambrian sediments.

7. A majority of crustaceans habitats are aquatic and they live in either marine or freshwater environments, but a few groups have adapted to life on land, such as terrestrial crabs, terrestrial hermit crabs, and marine environments.

8. The life cycle for different crustaceans starts from the nauplius stage, followed by the zoea larval stage and post-larval stage, and finally ends with the adult growth stage.

9. Ostracoda is an important example in crustacean.

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References

[1] Ortega-Hernández J. Making sense of 'lower' and 'upper' stem-group Euarthropoda, with comments on the strict use of the name Arthropoda von Siebold, 1848. Biological Reviews. 2016; **91**(1):255-273

[2] Chandra PP. Assessment of plant diversity in homegardens of three ecological zones of Nepal. Ecoprint; 2015;**22**:63-74

[3] Asvin P. Torres Ferran Palero Antonina Dos Santos Pere Abelló Edurne Blanco lexandra Boné and Guillermo Guerao: Larval stages of the deep-sea lobster *Polycheles typhlops* (Decapoda, Polychelida) identified by DNA analysis: Morphology, systematic, distribution and ecology. Helgoland Marine Research; September 2014;**68** (3):379-397

[4] Martin JW, Davis GE. An Updated Classification of the Recent Crustacea. Natural History Museum of Los Angeles County; 2001

[5] Jose J, Pillai L. Taxonomy and identification of commerciall important crustacean of india. Indian Council of Agriculture Journal. 2013. 169 p

[6] Martin RF, Randriandraisana A, Boulvais P. Ampandrandava and similar phlogopite deposits in southern Madagascar: Derivation from a silicocarbonatitic melt of crustal origin. Journal of Afrivan Earthscience. 2014;**2**

[7] Emerson MJ, Schram FR. The origin of crustacean biramous appendages and the evolution of arthropoda. Science. 1990;**4**:667

[8] Schram F, Vaupel K. Treatise On Zoology–Anatomy, Taxonomy, Biology the Crustacea Complementary to the Volumes Translated from the French of the Traité De Zoologie. Part B, Brill Leiden Boston: Koninklijke Brill NV; 2012;**9**:349

[9] Chen JY, Zhou GQ, Edgecombe GD, Ramsköld L. Head segmentation in early cambrian fuxianhuia: Implications for arthropod evolution. Science. 1995; **268**(5215):1339-1343

[10] Emmett Duffy J, Thiel M. Evolutionary Ecology of Social and Sexual Systems: Crustaceans as Model Organisms. Oxford; 2007

[11] Olesen J. Crustacean life cycles—developmental strategies and environmental adaptations. The Natural History of the Crustacea: Life Histories. Oxford University Press; 2018

[12] Jennifer Uhl has been writing professionally since 2005. She writes primarily for the web and has been published as a ghostwriter in "Tropical Fish Magazine" and "Entrepreneur." She is pursuing a Bachelor of Science in health care from Mira Costa College

[13] Gurney R. Larvae of DECAPOD Crustacea (PDF). London: Ray Society; 1942. pp. 1-306

[14] Mauchline J. Egg and brood sizes of oceanic pelagic crustaceans. Dunstaffnage Marine Research Laboratory. Scottish Marine Biological. Marine Ecology–Progress. 1988;**43**:251-258

[15] Torres AP, Santos AD, Alemany F, Massutí E. Larval stages of crustacean species of interest for conservation and fishing exploitation in the western Mediterranean. 2013

[16] Taylor PD, Wilson MA. Palaeoecology and evolution of marine hard substrate communities. Earth-Science Reviews. 2003;**62**:1-103

[17] Dworschak PC, Rodrigues SA. A modern analogue for the trace fossil Gyrolithes: Burrows of the

thalassinidean shrimp *Axianassa australis*. Lethaia. 1997;**30**(1):41-52

[18] Mayoral E, Mun F. Nuevosi datos icnotaxnomicos sobre *Gyrolithes* del Pliocene inferior de la Cuenca del Guadalquiver (Lepi, Huelva, Espana). Rivista Espanola de Paleontologia. 1998; **13**:61-69

[19] Lanés S, Manceñido M, Damborenea S. Lapispira: A double helicoidal burrow from Jurassic marine nearshore environments. In: Bromley RG, Buatois LA, Mángano MG, Genise J, Melchor R, editors. Sediment-Organism Interactions: A Multifaceted Ichnology. Tulsa: SEPM; 2007. pp. 59-78

[20] Gibert A. Toleration, Diversity and Global Justics. University of Pennsylvania; 2003. 233 p

[21] Haq BU, Boersma AB. Introduction to Marine Micropaleontology. North Holland: Elsevier; 1978. 376 p

[22] Brassier MD. Microfossils. In: Blackwell Publling Publishing. 1980

[23] Tinn O, Meidla T. Phylogenetic relationships of early middle Ordovician ostracods of Baltoscandia. Palaeontology. 2004;**47**:199-221

[24] Williams M, Floyd JD, Salas MJ, Siveter DJ, Stone P, Vannier JMC. Patterns of ostracod migration for the "North Atlantic" region during the Ordovician. Palaeogeography, Palaeoclimatology, Palaeoecology. 2003; **195**:193-228

[25] Siveter DJ, Vannier JM, Palmer D. Silurian Myodocopes: Pioneer pelagic ostracods and the chronology of an ecological shift. Journal of Micropalaeontology. UK: University of Leicester; 1991;**10**(**2**):151-173

[26] Bennett CE, Williams M, Leng MJ, Siveter DJ, Davies SJ, Sloane SH, et al. Diagensis of fossil ostracods: Implications for stable isotope based palaeoenvironmental reconstruction. Palaeogeography, Palaeoclimatology, Palaeoecology. Cambridge University Press; 2011;**305**:150-161

[27] Benson RH. The origin of the psychrosphere as recorded in changes of deep-sea ostracode assemblages. Lethaia. Wiley Online; 1975;**8**(1):69-83

[28] Ghafor IM, Mohialdeen IMJ. Early cretaceous microfossils associations (foraminifera, ostracoda, calcareous algae, and coral) from the Garagu Formation, Duhok Area, Kurdistan Region, Northern Iraq. Arabian Journal of Geosciences. 2018;**11**(15):1-206

[29] Taha ZA. The taxonomic, biogeographical, palaeogeographical and palaeoecological significance of the late ordovician Ostracod fauna of the Ellis Bay Formation, Anticosti Island, Eastern Canada [Ph.D. thesis]. United kingdom: Department of Geology University of Leicester; 2018

Chapter 2

The Robber Crab *Birgus latro* (Linnaeus, 1767)

Selvaraj Kumaralingam

Abstract

The robber or coconut crab *Birgus latro* (Linnaeus, 1767) is a terrestrial hermit crab. It is the largest—land living arthropod in the world. As far as India is concerned, distribution of this crab is restricted to Great Nicobar Island and South Sentinel Island in Andaman and Nicobar Archipelago. The crab divided into a front section (cephalothorax), which has eight legs, and an abdomen. The front-most pair of legs has large chelae (claws), with the left being larger than the right. The next two pairs, as with other hermit crabs, are large, powerful walking legs with pointed tips, which allow coconut crabs to climb vertical or overhanging surfaces.

Keywords: *Birgus latro*, Nicobar, India, robber crab

1. Introduction

The robber or coconut crab (*Birgus latro*; Linnaeus, 1767) is a terrestrial hermit crab. It is the largest—land living arthropod in the world. As far as India is concerned, distribution of this crab is restricted to Great Nicobar Island and South Sentinel Island in Andaman and Nicobar Archipelago. In the Nicobar Islands the species has been reported from Car Nicobar [1], Little Nicobar, Katchal, Camorta and Great Nicobar [2]. The crab separated into a visible section (cephalothorax), which has eight legs, and an abdomen. The next two pairs, as with other hermit crabs, are large, powerful walking legs with pointy tips, which allow coconut crabs to climb vertical or overhanging surfaces. The fourth pair of legs is slighter with tweezer-like chelae at the end, allowing young coconut crabs to grip the inside of a shell or coconut husk to carry for protection; adults use this pair for walking and climbing. The last pair of legs is very small and is used by females to tend their eggs, and by the males in mating. In the present study, the general ecology of coconut crabs in around great Nicobar is focused.

2. Methods

Coconut crabs are generally "easy to collect" and most often hand picking is very effective in intertidal zones, Crabs can be preserved wet in 6–10% formalin for further study. Field photographs by using the following taxonomic identification keys [3].

Coconut crab

Birgus latro (Linnaeus, 1767)

3. Results

3.1 Habitat

Coconut crabs are viewed as a standout amongst the most earthly decapods; the crab is an all-around adjusted loner crab, it is diurnal and night-time in propensities. Coconut crabs live alone in underground tunnels and shake hole, contingent upon the nearby territory. They delve their very own tunnels in sand or free soil. During the day, the creature remains concealed to diminish water misfortune from warmth. While resting in its tunnel, the coconut crab shuts the passages with one of its hooks to make the clammy microclimate inside the tunnel fundamental for its breathing organs They live solely ashore, coming back to the ocean just to discharge their eggs.

3.2 Size

This large sized crab grows up to 40 cm long and 22 cm wide (single sighting observed).

3.3 Feeding habit

Adult coconut crabs feed on fruits, nuts, seeds, and the pith of fallen trees, smaller worms, crustaceans and molluscs. The species is popularly associated with the coconut, and has been widely reported to climb trees to pick coconuts, which it then opens to eat the flesh.

3.4 Behavior

These Hermit crabs have a series of complex physical movements to communicate with other crabs in different situations. They are active at night which makes it difficult to see slight differences in body motion. They raise a single leg out and above the rest of their body as a warning to keep away.

3.5 Lifecycle

Coconut crabs are terrestrial animals whose eggs are hatched at sea. The female once her eggs have been fertilized by a male crab, will release her eggs into the sea with a new moon and a spring tide, when the humidity and temperature are right. The number of eggs she releases be a great as 138,000. The species' only dependence on the sea is for releasing eggs, which hatch in contact with seawater; the planktonic larvae then migrate onto land where they develop into long-lived adults [4]. And migrate to the shoreline with other terrestrial hermit crabs. The coconut crab reaches sexual maturity around 5 years after hatching. They reach their maximum size only after 40–60 years.

3.6 Distribution

South Sentinel Island and Nicobar Islands in the Bay of Bengal, Central Pacific Ocean, Ryukyu Islands, Coast of Tanzania, Tropics of Cancer and Northern and Southern limits of Capricorn mark, Australia, Madagascar and Mauritius Island.

4. Discussion

The population of this crab is dwindling due to habitat loss as a cause of coastal development and exploitation by human for food. Large populations exist on the Cook Islands especially Pukapuka, Suwarrow, Mangaia, Takutea, Mauke, Atiu, and Palmerston Island. The coconut crab *Birgus latro*, characterized as Data Deficient on the IUCN Red List [5], is the largest land crab. In worldwide conservation and management strategies have been put in place such as ban on the capture of egg-bearing females and avoid the hunting of non-egg-bearing adults having above carapace length 30 mm. In India the species protected under Schedule I category of Wildlife (Protection) Act, 1972. The Conservation needs of the coconut crab *Birgus latro* on the Nicobar Islands, India [6]. We recommend that more extensive surveys be carried out in all potential coconut crab habitats on the Andaman and Nicobar archipelago.

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References

[1] Hume AO. Contributions to the ornithology of India: The islands of the Bay of Bengal. Stray Feathers. 1874;**2**:29-324

[2] Daniel A, Premkumar VK. The coconut crab in the Great Nicobar Island. Journal of the Bombay Natural History Society. 1968;**64**:574-580

[3] Alcock A. Catalogue of the Indian Decapod Crustacea in the Collection of the Indian Museum. Kolkata, India: Zoological Survey of India; 1905

[4] Reese BS, Kinzie RA. The larval development of the coconut or robber crab *Birgus latro* (L.) in the laboratory (Anomura, Paguridae). Crustaceana Supplement. 1968;**2**:117-144

[5] Eldredge LG. *Birgus latro*. In: IUCN Red List of Threatened Species v. 2010.4. 1996. Available from: http://www.iucnredlist.org [Accessed: April 19, 2011]

[6] Patankar V, D'souza E. Conservation needs of the coconut crab *Birgus latro* on the Nicobar Islands, India. Oryx. 2012;**46**(2):175-178. DOI: 10.1017/S0030605311000408

Chapter 3

Scyllarid Lobster Biology and Ecology

Kari L. Lavalli, Ehud Spanier and Jason S. Goldstein

Abstract

The family Scyllaridae is the most speciose and diverse of all families of marine lobsters. Slipper lobsters are found in both tropical and temperate habitats with hard or soft substrates and at different depths, and exhibit a wide array of morphological, anatomical, and physiological adaptations. Among the 20 genera and at least 89 species constituting 4 subfamilies, only some members of 4 genera, *Thenus* (Theninae), *Scyllarides* (Arctidinae), *Ibacus* and *Parribacus* (Ibacinae), form significant fisheries because of their large size. While scientific information on these lobsters has increased considerably in recent decades, it is still limited compared with commercially valuable spiny and clawed lobsters, and is confined to a few key species. The present chapter presents the current available knowledge on the biology of scyllarids and attempts to point out where questions remain to help focus further studies in this important group.

Keywords: slipper lobsters, Scyllaridae, taxonomy, genetics, anatomy, physiology, ecology, life history, behavior, fisheries

1. Introduction

Slipper lobsters, family Scyllaridae (Latreille, 1825) have been known and described since the late 1700s and are considered part of the superfamily Palinuroidea that consists of spiny lobsters (Palinuridae), furry lobsters (Synaxidae), blind claw-footed lobsters (Polychelidae), and slipper or shovel-nosed or bulldozer lobsters (Scyllaridae) [1, 2]. The Scyllaridae are organized into four subfamilies (Ibacinae, Arctidiane, Scyllarinae, and Theninae) and comprise 20 genera with at least 89 extant species thus far recognized [3–6].

Only four genera—*Scyllarides* (Arctidinae), *Ibacus* and *Parribacus* (Ibacinae), and *Thenus* (Theninae)—form any kind of significant fishery because these individual species tend to be large in size [7]. Of these four genera, *Scyllarides* (Gill, 1898) has been studied extensively due to their large adult size, which makes them economically important; their worldwide geographical distribution in tropical and subtropical habitats; and their numerous species (14) [1]. Considerable knowledge is also available for species within the genus *Thenus* because of some relevance in certain fisheries as well as the success in rearing these animals in aquaculture [8]. Research on other species generally arises with overfishing of and a shift away from sister species (generally palinurids) and thus always lags behind exploitation, which is problematic for the creation of sustainable fisheries. Although small in size, lobsters of the genus *Scyllarus* often become a minor target for fisheries (e.g., [9, 10]).

The present review is an attempt to summarize the somewhat patchy information available in the scientific literature on scyllarids. In addition, expanding our knowledge on slipper lobsters may prove beneficial to humans in ways beyond providing a food source, given that large proteins recently isolated from *Ibacus novemdentatus* have displayed cytotoxic activity against human cancer cells [11].

2. Taxonomy, phylogeny and evolution

Lobsters were significantly more diverse in the Mesozoic, especially during the Triassic and Jurassic, than in the Cenozoic and Holocene. The Achelata appeared 391–351 million years ago (MYA), but did not diverge into the palinurid and scyllarid lineages until the Permian (~250 MYA) [12, 13]. Fossil remains of scyllarids date back to the mid-Cretaceous (100–120 MYA) [3], but are not well-represented since their fossils come mostly from low energy (shale, clay, ironstone) or lithographic (limestone) deposits [14–16]. Today's scyllarids live in different habitats (coral and sponge reefs, and medium to high energy environments) from fossil forms, but the sparse fossil record of this group makes it difficult to speculate on when their habitat shift occurred, although their major radiation began in the Late Jurassic and continued through the Holocene [14].

Slipper lobsters are closely related to the Palinuridae and Synaxidae, all of which comprise the Achelata; they share numerous characters, most notably their unique larval phase (i.e., phyllosoma) which separate the Achelata from all other Decapoda [3]. The plate-like antennal flagellum of slipper lobsters is a highly derived feature that is common to all 89 species and distinguishes them from the palinurids and synaxids which possess whip-like antennae. The Scyllaridae underwent considerable taxonomic revision from 1991 to 2002, mostly within the Scyllarinae, and now consist of 20 genera. The highest taxonomic diversity is among the smaller species [1, 17].

The subfamily Arctidinae consists of 2 genera and 17 species. These are some of the larger scyllarids. *Arctides* and *Scyllarides* species typically have a highly vaulted carapace, a three-segmented mandibular palp, and a shallow cervical incision along the lateral margin of the carapace. The subfamily Ibacinae consists of 3 genera, *Evibacus, Ibacus* and *Parribacus*, with a total of 15 species. In these species, the carapace is significantly dorso-ventrally compressed with a deep cervical incision along the lateral margin of the carapace. The mandibular palp is simple or two-segmented, in contrast to the Arctidinae. One genus, *Thenus*, and five species are recognized presently in the subfamily Theninae [5]. The Theninae display extremes: the body is highly flattened and their eye orbits are found at the extreme antero-lateral extent of the carapace. In contrast, the 52 species of Scyllarinae found within 14 genera all have vaulted carapaces covered with tubercles and their eyes are more medial in placement. Yet both Theninae and Scyllarinae lack a flagellum on the exopod of the first and third maxillipeds [18]. See **Figure 1** for representatives of these species and **Figure 2** for examples of scyllarid mouthparts.

The taxonomy of Scyllaridae is based mainly on the morphology of the adults and to lesser extent of that of their pelagic larvae, the phylosomas. Recently molecular genetic tools have been used to assess taxonomic and phylogenetic issues, and the main clades found within Scyllaridae are in agreement [13] with current taxonomy based on adult morphology [1, 19, 20] and recent molecular studies [5]. All subfamilies (Arctidinae, Theninae and Scyllarinae) are now considered monophyletic, except for the Ibacinae [5]; this contrasts with a more recent analysis [21] that concluded that the Scyllaridae are fully monophyletic. The Arctidinae appears to represent the earliest branching lineage during the evolution of this group [5], which corresponds to the fossil record. In addition, slipper lobsters have likely evolved from shallow (onshore)

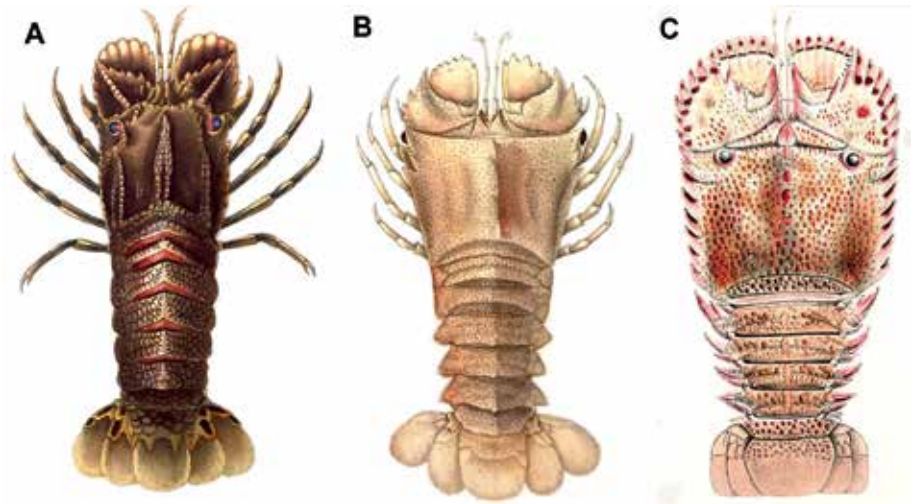

Figure 1.
Different forms of scyllarid lobsters. (A) Scyllarus arctus; (B) Thenus orientalis; (C) Parribacus antarcticus. A and B from Cuvier G. Le Règne animal: D'Après son organization, pour Sevir de base a L'Histoire Naturelle des Animaux, et D'Introduction a L'Anatomie Comparèe. Accompagnée de planches Gravées. Imprime chez Paul Renouard, Paris, France, 1837; C from Dana JD. United States exploring expedition during the years 1838, 1839, 1840, 1841, 1842 under the command of Charles Wilkes, U.S.N., Vol. XIII. Crustacea. C. Sherman, Philadelphia, 1852.

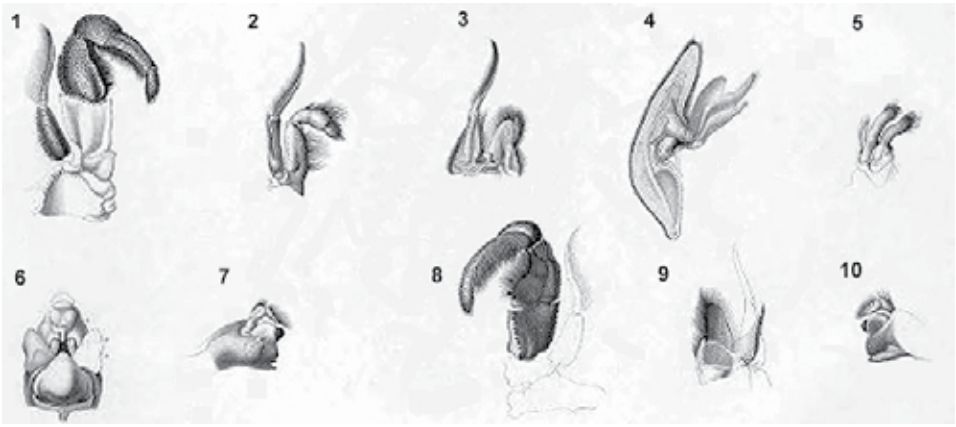

Figure 2.
Scyllarid mouthparts. (1) Aboral/ventral view of third maxilliped; (2) aboral/ventral view of second maxilliped; (3) aboral/ventral view of first maxilliped; (4) aboral/ventral view of second maxilla; (5) aboral/ventral view of first maxilla; (6) mouth; (7) aboral/ventral view of mandible; (8) oral/dorsal view of third maxilliped; (9) oral/dorsal view of first maxilliped; (10) oral/dorsal view of mandible. From Savigny, J-Cés. Iconographie des Crustacés et des Arachnides de l'Égypte. De l'Imprimerie Royale, Paris, France; 1805.

to deep water (offshore) species [5]. These same molecular tools suggest that two Atlantic species, *Scyllarus depressus* and *S. subarctus*, are a strongly supported clade with low genetic differentiation, indicative of a recent split into sister taxa [22].

3. Life history

The life history of scyllarids parallels that of palinurids and can be divided into a series of developmental phases. These lobsters typically begin their pelagic lives

as phyllosoma larvae (**Figure 3**), although some scyllarids (*Scyllarides aequinoctialis* [23, 24], *S. herklotsi* [25], *S. latus* [26], *Ibacus alticrenatus* [27] and *I. ciliates* [28] or *I. novemdentatus* [19]) hatch as a naupliosoma (pre-larva), a short-lived form lasting a few hours that bears only the first three pairs of cephalic appendages [29]. Abdominal appendages are typically absent or rudimentary in early phyllosomas, but appear in later stages [30]. Exopodites are found on all thoracic appendages of phyllosoma larvae until their metamorphic molt when they are lost from all but the first and second maxillipeds; here exopodites are retained and used for generating currents around the mouth region [31]. Scyllarid phyllosomas deviate from other decapod larvae in that they are missing a fully developed exopod on the third maxilliped and this may indicate a phylogenetic separation of feeding strategy [3].

The dispersal of phyllosomas varies among species and depends largely on whether the parental stock is found within lagoons formed by coral island barrier reefs or in deeper waters [32–36]. Those hatched in coastal lagoons tend to remain there, while those hatched in deeper water gradually move shoreward, such that final-stage phyllosomas are found much closer to shore [30]. Some phyllosomas undertake diel vertical migrations, but data are limited as to the extent of these migrations and the species-specific preferences for various depths [30, 37] as well as the efficacy of their swimming behavior. It is likely that smaller instars vertically migrate less than later, larger instars [35] and may use passive transport by occupying vertical strata that move them in specific directions [30]. Some phyllosomas even travel attached to the aboral surface of jellyfish medusae or siphonophores [38–41], which may affect larval dispersal or allow them to remain relatively near shore [29, 30]. Understanding of phyllosoma behavior and dispersion has been challenged by the ability to correctly identify species; however, recent use of molecular genetics and DNA barcoding is improving the ability to make species identification possible in the field [42, 43].

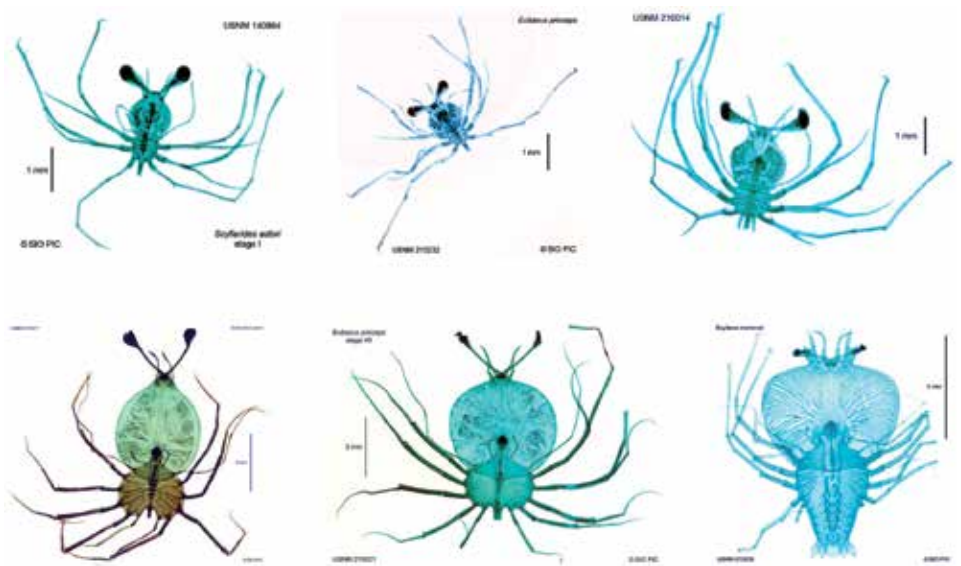

Figure 3.
Various stages of scyllarid phyllosoma larvae. Top, early stages of Scyllarides astori and Evibacus princeps. Bottom, later stages of S. astori, E. princeps, and Scyllarus martensii. From the Martin Wiggo Johnson Phyllosoma slide collection of the Scripps Institute of Oceanography Pelagic Invertebrate Collection website (https://scripps.ucsd.edu/collections/pi/overview/collection-databases-zooplankton-guide/m-w-johnson-lobster-phyllosoma-slide).

Phyllosomas are raptorial feeders, using their pereiopods to grasp onto food items, which are then shredded by the maxillipeds and masticated by molar processes of the mandibles [44]. Mostly fleshy foods are ingested; such food types are more readily available in coastal waters than in offshore, oligotrophic waters [29, 30, 45]. Some scyllarid phyllosomas have been observed clinging onto or "riding" the medusa stage of some gelatinous zooplankton. For example, a recent report of a videotaped scyllarid lobster phyllosoma swimming while dragging a prayid siphonophore behind it suggests that gelatinous forms may serve as a critical food and/or defense against predation (by ingestion of the nematocysts) and refutes the idea that hitching a ride on these organisms is energy-saving due to passive transportation [41]. Recently, molecular methods using the central domain of the 18SrDNA gene have identified food items of some species of scyllarid and palinurid phyllosomas and suggest that these forms feed on appendicularians, salps, and cnidarians [46]. Ctenophores fed to phyllosomas of *Thenus orientalis* are accepted readily and provide nutritional support [47] and similar results were obtained with the phyllosomas of *T. australiensis*, *Ibacus novemdentatus*, *I. ciliatus* fed on jellyfish [48–51]. Some species of wild phyllosomas were found to contain cnidarian tissue in their hepatopancreas and feces, and these phyllosomas seem capable of encapsulating nematocysts [52] suggesting that these larvae utilize jellyfish as a food source. Few studies have examined exactly how phyllosomas consume jellyfish, but one possible mechanism is for phyllosomas to cling onto the exumbrella, feed on tentacles or oral arms first, and then consume the exumbrella [48, 53]. Phyllosomas riding on jellyfish manage to groom and clear mucus extruded by jellyfish to dampen microbial growth on their bodies [54].

The final-stage phyllosoma molts into the highly specialized nisto (see **Figure 4**), or post-larval stage, which, like their spiny lobster (pueruli) and clawed lobster (post-larvae) counterparts, utilize surface waters to swim toward benthic habitats to settle. Nistos are neither completely planktonic nor completely benthic—they are caught in plankton tows demonstrating that they are pelagic at least part of the time [29]. In many species of scyllarids, the nisto appears to bury into soft substrates during the day and swim actively at night; some species even change coloration daily between these two habitats to remain cryptically colored in both environments [29]. Some scyllarid nistos are excellent swimmers (using their abdominal pleopods), while others are poor swimmers; some are also capable of executing tail flips (backward swimming) as a means of escape [55]. These swimming differences may exist due to marked differences in the size of pleopods among different species [56]. However, this suggestion has not been adequately tested.

As with spiny lobster pueruli, the nisto appears to rely on energy reserves, rather than to actively feed [30], although the structure of the proventriculus is transitional between the phyllosoma and the juvenile [57] which suggests that it can process and sort food particles at this stage of development. The nisto also bears a cardio-pyloric valve that divides the anterior and posterior cardiac chambers, but lacks a gastric mill. Thus, if food is consumed by the nisto, it is likely soft and processed mainly by the mouthparts prior to ingestion [57]. Nistos appear similar in form to juveniles and bear the derived feature of flattened antennae, but are transparent instead of being reddish-brown. Their abdominal pleopods still bear swimming (natatory) setae [58] to aid in transitioning them from the pelagic to the benthic realm.

Juvenile life history of scyllarids is lacking for all species except those that have been successfully reared in culture (e.g., *Thenus* species [59] or *Ibacus* species [49, 60, 61]). This primarily is the result of a problem in sampling and not knowing where juvenile grounds lay. For example, in *S. latus* no live juvenile or nisto of the

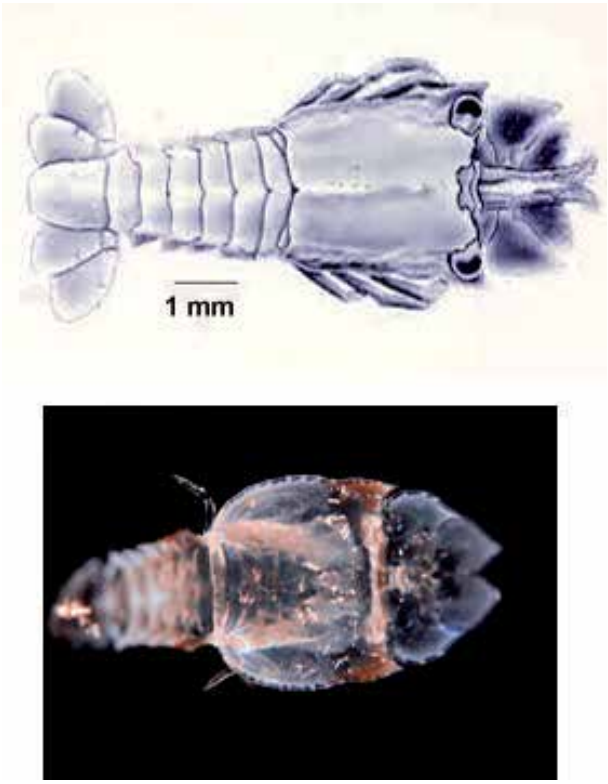

Figure 4.
Nisto of Scyllarus americanus (Top). From Ref. [23]. Open access: https://archive.org/details/larvaldevelopmenoorobe. Nisto of unidentified scyllarid species in Florida waters (Bottom). Photo by Casey Butler.

commercially exploited Mediterranean slipper lobster, *Scyllarides latus* (Latreille, 1851), had ever been sampled despite ample information available on the ecology and behavior of adults of this species [62]. Museum surveys of invertebrate collections provided a small specimen of *S. latus* (36 mm carapace length (CL)) collected in 1987 with a 20 mm mesh scientific trawl net at depth of 450–700 m on a soft and muddy bottom at least 40 km offshore of Livorno [63]. Another specimen, even smaller (11.7 mm CL), was collected in Reggio Calabria, southern Italy, in the early 1900s at a depth of >850 m and deposited in the Zoological Museum of Turin. This early scyllarid juvenile, likely a recent benthic recruit, suggests that the larvae drift large distances before settling as nistos in deeper waters with muddy habitats where they are possibly protected against the more numerous inshore predators. They then migrate as larger juveniles or sub-adults to inshore habitats [63]. Similar suggestions have been made for other scyllarids. A recent study [64] found *Scyllarus* sp. in the guts of deep sea fish which suggests that nistos are settling in deep waters. *Ibacus* juveniles appear to migrate shoreward from offshore waters to recruit into adult grounds [61]. Juveniles appear to occupy a different spatial niche from adults and are far more cryptic than adults because few individuals are found that are smaller than 20 cm TL [65, 66]. To obtain sufficient numbers of small individuals, specific sampling techniques must be developed which target the juveniles, which may prove difficult if many of the species have juvenile development in deep, oceanic waters. The exceptional discovery of a juvenile form of scyllarid in the old museum collection of Turin [63] emphasizes the importance of comprehensive surveys of crustacean collections, even old ones, in search for scyllarid life stages.

Gaps in life-history make growth rate determination difficult in most species, except for those that can be cultured with high survival rates or from grow-out studies when sufficient juveniles have been captured. Juveniles of reared *S. nodifer* take ~18 months and 9–10 molts to reach adult size [67]. Other fast growing species include *Ibacus* spp. that reach sexual maturity after four to six molts [68]. Cultured *Thenus orientalis* take about 400 days (19 molts) to grow to a size of *c.* 250 g. [69]. In contrast, 7–8 years are necessary for juvenile *S. astori* to recruit fully into the adult population [65]. Hence, from what little data we have on juvenile life history, it appears that many, but not all, of the commercially important scyllarids are capable of rapid growth.

Arctidinid adults (e.g. *Scyllarides* spp.) are typically large and tag–release studies suggest that adults molt annually (*S. latus*, [70]), although data from *S. astori* populations suggest that molts occur every 18–24 months [65, 66]. Molting typically occurs at night and in cooler to warmer months [71–73]. Softening of the old exoskeleton starts some 10–22 days pre-molt, with hardening being complete 3 weeks post-molt. The entire process takes approximately 7 hours, with lobsters remaining shelter-bound for 5–9 days post-molt [74]. Slipper lobsters do not appear to consume their exuviae since these are generally left outside of shelters [72]. Sex ratios are close to unity in those species that have been adequately sampled (*S. latus*, [26, 72]; *S. astori*, [65]). In some species, mean CL is larger for females than for males (*S. latus*, [26, 74]), while in others, males exceed females in size (*S. astori*, [66]). Shortly after mating, females extrude a large number of eggs (conservative numbers range from 24,710 to 356,000), based on TL of the individual, with those eggs ranging from 0.6 to 0.7 mm diameter [26, 66, 75–77]. In some species, spawning occurs twice a year [76]. Such high fecundity rates may be an adaptation to oceanic loss of larvae and variable recruitment of nistos due to cyclic changes in oceanic climate [29]. Eggs are brooded for 2–8 weeks before release over a number of days (*S. latus*, [74, 78]). Ovigerous females are more commonly sampled in cooler months, but not warmer months [66]. There is some evidence that females may return to inshore reefs in the autumn earlier than males and leave sooner after shedding eggs in the mid-summer, possibly to maximize thermal regimes for developing embryos [79]. Most species appear to move to colder, deeper waters when inshore water temperatures rise steeply in the summer or, for those species that remain in lagoons, stay at locations where thermal regimes are less than 25°C [65].

Ibacus sp. adults rarely exceed 20 cm TL [1] and are thus smaller than the adults of subfamily Arctidinidae. Sex ratios of all four Australian commercially caught species of *Ibacus* are approximately 1:1. Males are smaller than females because they molt less frequently after attaining sexual maturity. Mating occurs when the female is hard-shelled. Fecundity is much lower than in members of the subfamily Arctidinidae and it is highly variable both within and among the four species of *Ibacus*. It increases with the size of the animal [68]. Egg incubation times have been estimated to vary between approximately 2–4 months and are likely to be temperature dependent with longer incubation in cooler water [61]. Molt frequencies of captive lobsters suggest seasonal molting but wild, tagged lobsters were caught repetitively in consecutive years without having increased in size [68]. Growth models for *I. peronii*, suggest the potential for this species to live for more than 15 years with a maximum size reached after 5–8 years [80].

Very little information is available on adults of *Parribacus* spp. and what does exist is mainly focused on *P. antarcticus*. Two captured females of this species bore evidence of reproductive activity during summer (July) [81].

In *Thenus* spp. growth is quite rapid with 80% of maximum size reached by 2 years of age. Females appear to attain larger sizes than males as evidenced by fishery sampled size ranges in both Indian and Australian waters [82]. Increased

abdominal dimensions likely explain the greater weight of females, while maximization of reproductive efficiency via larger size and the ability to carry more eggs explains the greater mean size of females [83]. However, the two sexes eventually grow to a similar size [84]. Differences between Indian and Australian populations of thenids may reflect differences between sub-species or even between different species in view of the recent taxonomic revision of the genus [4].

In the *T. orientalis* fisheries off India, sex ratios are 1:1 [85], but off Australia they are skewed toward males [83] with a ratio of 0.57. In contrast, *T. indicus* sex ratios are at 1:1 throughout the year [83]. As with all scyllarids, fecundity of thenids scales with length. Various studies in India and Australia show at least two annual spawning periods [83, 84]; however, only a single spawning period was reported for *T. orientalis* off the Tokar delta in the Red Sea [84].

Adults of the subfamily Scyllarinae are usually small and information is very limited regarding growth and reproduction. *Scyllarus arctus* appears to have a continuous reproductive period where females can spawn up to three times per year [10]. The sex ratio is skewed toward females and mean size is larger in females.

4. Genetics and population continuity

The developmental period for scyllarid phyllosomas is far more variable than that for palinurids, and can last from a few weeks to at least 9 months [29, 30]. Lengthy duration of the larval period likely leads to wide oceanic dispersion and, ultimately, connectivity of geographically distant subpopulations resulting in panmixia in adults. Molecular tools are just starting to be used to examine population structure of individual species. In one such study, *S. latus* collected in 2 locations in the Western Mediterranean and 13 locations in four regions in the NE Atlantic, including Southern Portugal and the Macaronesian archipelagos, revealed genetic homogeneity in *S. latus* across all regions [86]. More such studies in other species are needed to understand the population genetics of scyllarid species.

5. Behavior

Except for *Scyllarides latus* and *Thenus orientalis*, both of which are readily held in laboratory settings, behavior of most slipper lobsters has not been well studied. In addition, the sensory modalities used for behaviors are not well understood as they are in nephropid and palinurid lobsters [87].

5.1 Feeding behavior

Feeding behavior of adults is dependent on the structures with which lobsters can capture, manipulate, and process their food and differs with life history stage as mouthparts, pereiopods, and the proventriculus gain substance and size. Feeding habits, primarily for the adults of *T. orientalis* [88] and *I. peronii* [89], and *Scyllarides* spp. [90] are known.

As in clawed and spiny lobsters, the esophagus of slipper lobsters is short, presumably to allow for rapid ingestion [57]. This structure leads into the proventriculus, which is divided into the anterior cardiac stomach and the posterior pyloric stomach. The gastric mill of slipper lobsters is smaller and less calcified [88] likely due to the diet specialization that has occurred in slipper lobsters—that of primarily consuming bivalve flesh, or other fleshy items. Food proceeds from the cardiac stomach to the pyloric stomach through a cardio-pyloric valve, which lacks

the spines and accessory teeth seen in other decapods [88]. Dense mats of setae in the pyloric stomach provide filtering of semi-digested food particles with only the smallest particles entering from the cardiac stomach and exiting into the digestive gland. Larger particles are passed into the midgut caecum and hindgut [88]. Little is understood about the digestive enzymes involved in food breakdown [57].

Many slipper lobsters (e.g. *Scyllarides* spp.) are bivalve specialists and these have evolved the ability to use the nails of their pereiopods to shuck bivalves [90, 91]. During the feeding sequence, slipper lobsters typically probe the outer valves with their antennules, as though "smelling" and assessing the shell for its possible value [92]. They then pick up and hold the bivalve with either the first, third, and fourth or second, third, and fourth pairs of walking legs, using the dactyl tips of the first or second walking legs to repetitively probe the valve edges [92]. The dactyl tips eventually wedge into the shell edge and then push in further and further to open the valves; this process is known as "wedging" [90]. Once the valves are opened enough to fully insert one pair of pereiopod dactyls, another pair of walking legs (second or third) are used to cut the mantle tissue along the pallial line. The lobster then uses a back-and-forth "scissoring" type motion to increase the opening angle to reach the adductor muscles [92]. The second pair of walking legs cut the adductor muscles, so that the valves open freely. With the valves open, the meat is repetitively scraped out of the valves and passed directly to the third maxillipeds [90, 92]; see **Figure 5**. Until the flesh is actually passed back to the third maxillipeds, the antennules make repeated downward motions to probe inside the valves, to touch the flesh, and to touch the shell as the legs scrap the flesh from it; it is likely that the antennules act as a dual "smell" and "taste" sensory modality due to the damage to pereiopod setae from the process of shucking [92].

While bivalves are a preferred food source, slipper lobsters are also known to consume sea urchins, crustaceans, sponges, gastropods, barnacles, sea squirts, algae (*Ulva* spp.), and fish [66, 93]. Gut contents of commercially fished *T. orientalis* in India included a high proportion of mollusks (27.7%) followed by bottom sediments (24.1%), fishes (22.9%), crustaceans (10.7%), polychaetes (4.2%) and miscellaneous food items (10.4%) [84]. Scallops, goatfish and shrimps were always consumed when offered under laboratory conditions [83]. Thus, based on stomach contents and laboratory behavior, *T. orientalis* appears to be an opportunistic,

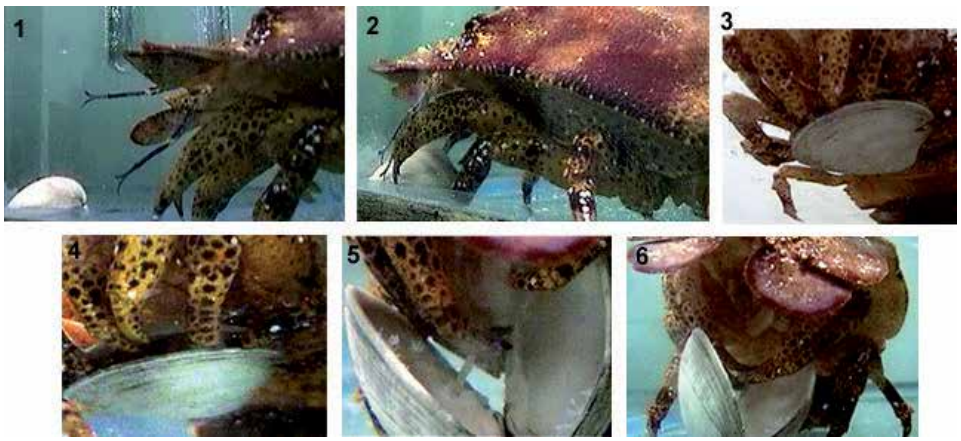

Figure 5.
The feeding sequence of S. aequinoctialis. (1) Lobster approaches bivalve with antennules flicking and sampling odors; (2) pereiopods grab bivalve while antennules "taste" it to assess if feeding sequence will continue; (3) "wedging" of pereiopods into closed valves; (4) probing and "shucking" of the valves, while cutting adductor muscles; (5) close-up of pereiopods ripping adductor muscle; (6) scraping of flesh out of bivalve and delivery to mouthparts.

omnivorous, benthic feeder that burrows in soft and sandy mud, engulfs sediments consisting of sand and mud, and then preys on organisms that it encounters in this way [84].

5.2 Sheltering behavior and substrate preferences

Adult specimens of *Scyllarides* spp. are camouflaged to a certain extent due to their flattened morphology and coloration that blends into hard substrates (e.g. [72, 94]). However, in the brightly illuminated water of their shallow habitats, this camouflage provides only limited concealment against diurnal predators. Thus, most are nocturnal, foraging at night and sheltering during the day ([66, 95, 96] for *S. astori*; [72] for *S. latus*). A more recent set of lab studies documented that *S. latus* is more active at higher temperatures, and demonstrated that warming water temperatures elicited markedly longer movements [97].

Gregarious sheltering has been noted for *S. latus* (Spanier, personal observation) but predation studies at field sites demonstrate that grouping does not decrease per-capita predation rates on individuals within the group. Grouped lobsters suffer an equal rate of predation as lone animals and gain only a small advantage of time, as predatory attack patterns are less focused when lobsters are grouped [98]. Reports of gregarious behavior also exist for *S. nodifer* [99], but nothing is known about the function of such behavior.

The adults of many species are found on hard and soft substrates (**Figure 6**). *Scyllarides* species sampled both on hard (rocks, caves, coral heads) and soft substrates often result from circumstances where lobsters that usually shelter in hard substrates were collected in soft substrates during their short and long term movements, but some species such as *Scyllarides elisabethae*, *S. nodifer*, and

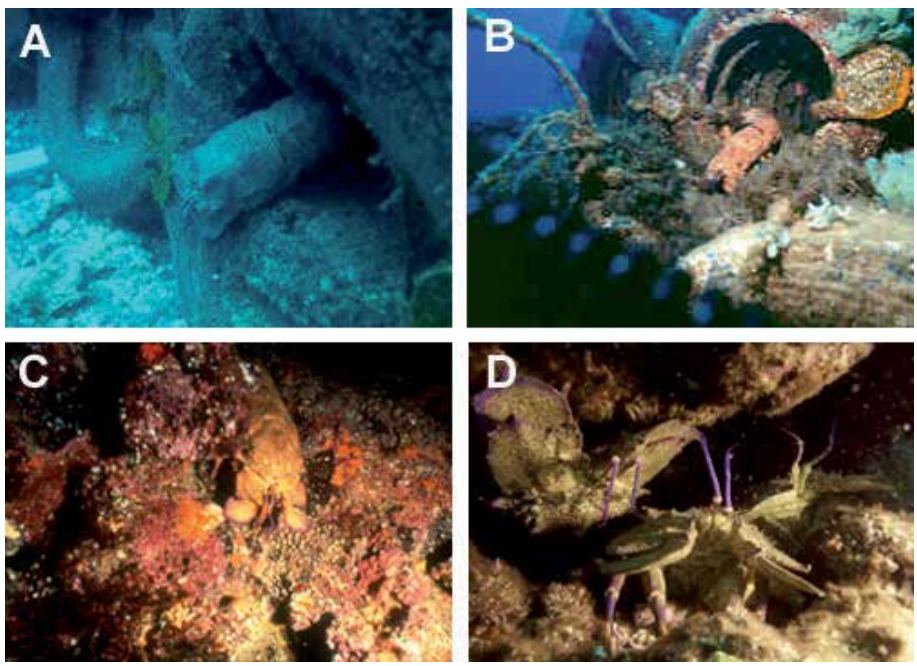

Figure 6.
Scyllarides latus in artificial reef structures (A, B) and natural rock outcropping (C, D). In natural outcroppings and large openings in artificial reefs, they typically co-habit space with other conspecifics (B, D). Photographs by Stephen Breitstein.

S. aequinoctialis are only found in mud or sand [1, 99, 100]. *Parribacus* species also inhabit hard substrates (corals structures, caves) or are found in sandy bottom [1, 81]. All five species of *Thenus*, and eight species of *Ibacus* inhabit relatively soft sandy or muddy substrates [4, 68] and are well-adapted for digging into the substrate in terms of their morphology as well as their behavior. *I. peronii* spends most of the day underneath the sand [101] and both *T. indicus* and *T. orientalis* spent daytime hours buried in sediment with only eyes and antennules exposed [83], but were nocturnally active, with clear peaks in activity at dusk and just prior to dawn.

5.3 Predators and antipredator behavior

The response of slipper lobsters to predator attack (e.g., by gregarious triggerfish) has been well studied [79, 98, 102–108] and consists of three strategies, two of which are typically executed in sequence: (i) the "fortress strategy" in which the animal grasps the bottom and attempts to outlast its attacker's motivation to penetrate its hard shell (described in [107]); (ii) the "swimming escape" response (described in [102, 105–107]); and (iii) remaining sheltered in dens [79, 103]. Lacking claws (like *Homarus* spp.) or long spinose antennae (like spiny lobsters; see [109–112]) with which to fend off swimming predators, slipper lobsters have developed a shell that is thicker and more durable to mechanical insult than clawed or spiny lobsters [107]. They use their short, strong legs to grasp the substrate and resist being dislodged [105, 106] (see **Figure 7**), and if this fails, they are exceptionally deft swimmers capable of evasive maneuvers [102]. Also they may suddenly change the direction of their swimming, presumably to confuse the chasing predator. This is an energetically costly response to a threat and is generally used as a last resort. Slipper lobsters may match the energy invested by clawed lobsters in claws and spiny lobsters in antennae by increasing only moderately the thickness of their shells and bettering their swimming escape behavior [107].

Slipper lobsters that live in complex substrates also display a variety of shelter-related behaviors that provide a third highly effective survival strategy [105]. By combining nocturnal foraging with diurnal sheltering, as well as carrying food to their shelters for later consumption, slipper lobsters may fully minimize their exposure to diurnal predators. The tendency for cohabitation with conspecifics (as seen in *P. antarcticus* [1, 81] or *S. latus* [98]) may be adaptive because of confusion effects (which lobster to target), alerting earlier to predators due to higher levels of "prey vigilance", or being concealed among conspecifics ("dilution effect" *sensu* [113]; see **Figure 7**). If these tactics fail, their thick carapace effectively blunt cracks [107, 114, 115] and may buy them extra time for escape when attacked.

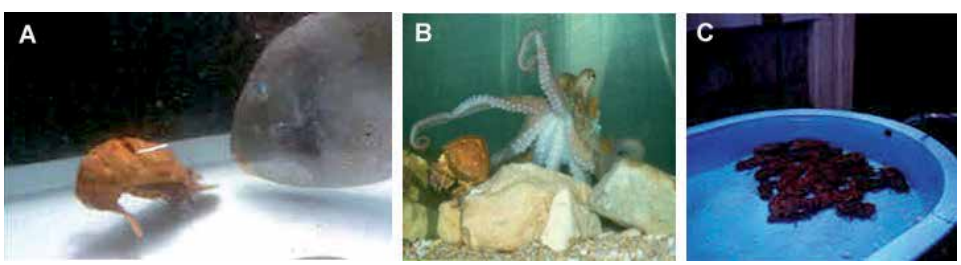

Figure 7.
Anti-predator responses of Scyllarides latus. (A) Tail flip in response to on-coming threat by triggerfish; (B) wedging into rocks in response to a predator (such as octopus) that can grip; (C) gregarious behavior in absence of shelter where each individual is concealed among other conspecifics. Photographs by Ehud Spanier.

Very little is known about the antipredator behavior of soft bottom species. Fully buried *Thenus* spp. are entirely concealed except for the eyes and antennules [83]. *Ibacus* spp. also are found on soft bottom substrates and are known to bury into those sediments, much in the same manner as *Thenus* spp. [68, 101] presumably also for concealment.

Besides triggerfish, spotted gully shark (*Triakis megalopterus* (Smith, 1849) have been reported to feed on *S. elisabethae* in South Africa [116], groupers (*Epinephelus* and *Mycteroperca* spp.) have been reported as predators of adult and juvenile *S. latus* [26] and *S. arctus*, *S. aequinoctialis*, and *S. nodifer* [56, 117]. Combers (*Serranus* spp.) and rainbow wrasse (*Coris julis* Linnaeus, 1758) apparently prey on juvenile *S. latus* [26]. Juvenile *S. aequinoctialis* were found in the gut of a large invasive alien red lionfish (*Pterois* spp.) in Belize [118].

5.4 Mating behavior

Most information on reproductive behavior comes from laboratory observations. Unlike clawed lobsters where mating usually occurs shortly after females molt, scyllarids are more similar to palinurids in that mating and molting are separate and unrelated events, although in the hooded slipper lobster (*S. deceptor*), copulation follows molting [119]. The general decoupling of molting and mating is largely due to males supplying females with external spermatophores that females use within hours or a few days. Nevertheless, some differences exist among the different subfamilies and those are summarized here. Males of *Scyllarides* spp. produce white, gelatinous spermatophores, which they carry around on the base of their fourth and fifth pereiopods ([74]; Spanier, personal observations) and transfer to females. In some species, females have been observed carrying spermatophores externally 6–10 days prior to egg extrusion (*S. latus*, [26, 74]), while in others, the lack of observable spermatophores prior to egg extrusion has led to a belief that the spermatophore is stored internally and fertilization is internal (*S. nodifer*, [55]; *S. squammosus*, [120]). Females of many species can spawn multiple broods in a season due to short brooding periods, and these broods are usually carried during spring and summer months. Only in *S. latus* have both eggs and spermatophores been observed simultaneously [74].

Male *Thenus* spp. do not appear to deposit a persistent spermatophoric mass in the process of mating [83]. Soft, non-persistent masses were observed [121] for *T. orientalis* and females oviposited within 8 hours post-mating and lost the spermatophore within 12 hours. No courtship behaviors or acts of mating have been witnessed in *Thenus orientalis* or *T. indicus* during 2000 hours of remote video observation [83], so it assumed that mating rituals are very simple. In *Ibacus* spp. spermatophoric masses were persistent, gelatinous, and opaque white in color, and were deposited in two elongated strips, approximately 20–30 mm long, close to the genital openings of the female [68]. Fertilization is likely external and occurs relatively soon after mating.

From the very limited information available on *Parribacus* spp., it appears that spermatophores are persistent even after spawning and new spermatophores are deposited atop old ones [81].

In *Scyllarus* spp. males deposit two jelly-like strings of spermatophores ventrally from the base of the fifth pereiopod to the second abdominal segment; these are used within hours to inseminate eggs and any remaining sperm mass degrades quickly [10]. Females are capable of multiple spawning events per year, but the number depends largely on environmental conditions; members of the same species may produce three broods in one area, but only two in another. This flexibility in

reproduction may prove advantageous when thermal regimes are favorable for rapid gonadal maturation, shorter incubation periods, or rapid larval development [10].

5.5 Movement patterns

Slipper lobsters movements consist of either slow, benthic walking used for daily nomadic movements within a small home range and for seasonal migrations from shallow inshore waters to deeper offshore waters or swimming movements that are used for escape or vertical migratory movements. Daily activity patterns suggest that slipper lobsters have endogenous clocks that provide for circadian rhythms with higher locomotor periods during night hours [122]. Tagging studies of *S. latus* off the coast of Israel confirm the slow, benthic walking patterns: local movements within a home range, presumably to forage and migratory offshore movements [79]. While residing inshore (February to June in the south-eastern Mediterranean), lobsters make short-range movements from reef shelters to forage and 71% return to these reef shelters. However, lobster numbers decreased in the reef shelters, ultimately decreasing to zero in summer months (June through August), and lobsters did not return to the reef until the following winter when their numbers peaked in the spring. This suggests a migration offshore that would correspond to increased water temperatures inshore [79]. Similar tagging studies off Sicily showed no such migratory movements [70]. *Scyllarides squamosus* also appears to make no long-range migratory movements, with mean distances moved from tagging location by most (97.2%) individuals being <1 km over a 5 year period [123].

Mobility of *Thenus* spp. in Australia also has been examined through use of tag and recapture studies and monitoring of commercial catch levels [83, 84]. *Thenus* spp. tend to be very mobile and capable of moving large distances, but because their movements lack any kind of pattern or directionality, they are not likely to be migratory. Likewise, *Ibacus* spp. exhibit nomadic movement patterns that have no directional patterns [124].

Swimming behavior constitutes a form of locomotion in which a single "appendage"—the abdomen—produces thrust by a combination of a rowing action and a final "squeeze" force when the abdomen presses against the cephalothorax [125]. Although the tail-flip response is known in adults and juveniles of all three major taxonomic group of lobsters, it is best developed in slipper lobsters.

The hydrodynamics of swimming in slipper lobsters has been studied in *Ibacus peroni*, *I. alticrenatus* [101, 126–128], *Thenus orientalis* [83, 126, 127, 129] and *Scyllarides latus* [102, 130, 131]. *S. latus* uses a "burst-and-coast" type of swimming in response to a threat. This burst-and-coast swimming consists of large amplitude movements of the abdomen followed by periods of powerless gliding. Acceleration can reach top velocities of three body lengths per second while deceleration during gliding decreases to velocities of less than one body length per second. Escape swimming is of short duration used only in emergencies to get to safety, as it requires considerable energy. The flattened second antennae of *S. latus* (mistakenly called "shovels" or "flippers"), with their movable joints, serve as stabilizers or rudders to control the swimming movement [130]. This adjustment in lift via the second antennae is also seen in *Ibacus* spp. and *Thenus* spp. [127].

In *Thenus* spp., there are two distinct forms of swimming: a locomotory form that is characterized by a slower speed (average of 29 cm s^{-1}) and the absence of explicit stimulus, and escape swimming which is much faster (average of 1 m s^{-1}), similar to that seen in *S. latus*, and always caused by direct stimulus or threat [83, 129].

In locomotory swimming, the aerofoil body shape generates lift as the abdomen thrusts downward; drag is reduced by all pereiopods being extended anteriorly [127]. Lift height was controlled by the second antennae and each flexion helped to maintain the animal above the sediment [127, 129]. In comparison, escape swimming always consisted of an abdominal flexion that was proportional to the magnitude of the stimulus. While *Ibacus* spp. can tail flip, it does not do so in response to a sudden threat, but seems to be related more to a righting response when the animal is flipped over [128].

6. Diseases

There are only a few reports on diseases or parasites of slipper lobsters, in general, and of specific species in particular [132, 133]. This limited information is usually focused on commercial species and those that have potential in aquaculture. *Scyllarides* specimens die while being held in the laboratory from unknown causes. Halacarid mites, *Copidognathus* spp., cause tissue necrosis in the gills of the *P. antarcticus* [133]. Aquaculture of *Thenus* spp. and *Ibacus* spp. will require more knowledge on pathogens of these species since the phyllosomas are very susceptible to microorganisms in the water column [69, 134]. There are also reports of parasites in adults. For example, a new species of parasitic copepod, *Choniomyzon inflatus* n., has recently been collected from the external egg masses of the smooth fan lobster *Ibacus novemdentatu* [135]. The Gram-negative *Vibrio* causes mass mortality during hatchery production of phyllosoma larvae and also affects their live feed of *Artemia* nauplii. Filamentous bacteria (*Leucothrix* sp.) and protozoans (such as *Zoothamnium* spp., *Vorticella* spp. and *Acinata* spp.) can also biofoul the phyllosomas and cause mortality [8]. Traditionally, a number of antibiotics as well as other chemicals have been heavily used for controlling bacterial colonies in the rearing water. Alternative methods are the use of ultraviolet light (UV) and ozone (O_3) sterilizers [8, 69].

7. Environmental effects and conservation

Overfishing, climate change, and habitat degradation are the main reasons for the drastic decline of marine populations over the past 30 years [136]. The effects of overfishing characterize many populations of commercial slipper lobsters and result in decreases in exploited stocks in the last few decades. Some species of slipper lobsters, formerly ignored, are now targeted due to the decline in other species (e.g., spiny lobsters) especially around the waters off Australia, Hawaii, India, the Galápagos Islands, and the countries surrounding the Mediterranean Sea. As a consequence, slipper lobsters have rapidly decreased in stock abundance to the point that local fisheries have collapsed [7]. Regulations established that try to protect these populations may have unexpected negative effects. For example, the prohibition against landing ovigerous females of *Scyllarus arctus* in NE Spain has biased the fishery toward males [10], which then affects natural sex ratios, opportunities for females to find mates, and ultimately population structure. Protected natural reserves/no-take zones can, to a certain extent, help rectify these effects [137], but require governmental action and policing. A fully protected, natural reserve off the northern Mediterranean coast of Israel has demonstrated significantly higher numbers of female and male *Scyllarides latus* compared to a control area with the same characteristics [138]. The specimens in the reserve were also significantly larger than those in the control, non-protected area.

Instead of regulations that may have unintended consequences or the creation of natural reserves that require political will, policing, and industry buy-in, targeted fishing moratoriums may also help to rebuild stocks. For example, depleted stocks of *S. elisabethae* recovered during a six-year moratorium from fishing and trapping off eastern South Africa [139]. However, despite years of protection, populations of *S. squammosus* in the Northwestern Hawaiian Islands, have failed to recover [140]. Possible factors that may limit population growth and recovery, include: climate change, Allee effects, and interspecific interactions. Community changes that come from overfishing of coral reef fauna might have broad and lasting results; once lost, valuable resources and ecosystem services may not quickly rebound to pre-exploitation levels and may have cascading effects on the larger fauna that rely on these resources [140]. Projected climate change impacts on the distribution of coastal lobsters, including a synthesis of 68 slipper lobsters species, suggest negative changes in diversity in areas of high commercial fishing due to habitat loss [141]. Such changes are expected to be particularly dramatic in the tropics, with species projected to contract their climatic envelope between 40 and 100% [141].

8. Conclusions

Although slipper lobsters represent the most speciose group of lobsters and have been exploited in targeted or by-catch fisheries, they have been and continue to be poorly studied compared to the less speciose but more popular clawed and spiny lobsters. Lack of knowledge of basic biological features such as life history, behavior, physiology, and disease does not bode well for the long-term health of populations especially when most scientists expect dramatic climatic changes to impact oceanic habitats and community structure. Given that these lobsters represent a potential food source for an ever-growing human population, it would be beneficial to understand much more about these lobsters with targeted studies, supported by governmental agencies, much as we saw for clawed and spiny lobsters nearly 40 years ago.

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References

[1] Holthuis LB. Marine Lobsters of the World. FAO Fisheries Synopsis No.125. Vol. 13. Rome: FAO; 1991

[2] Lavalli KL, Spanier E. The Palinura. In: Forest J, von Vaupel Klein JC, editors. The Crustacea, Traite de Zoologie 9A—Decapoda. Koninklijke Brill: Leiden, Boston; 2010. pp. 426-532

[3] Webber WR, Booth JD. Taxonomy and evolution. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster, Crustacean Issues 17. New York: CRC Press, Francis & Taylor Group; 2007. pp. 3-21

[4] Burton TE, Davie PJF. A revision of the shovel-nosed lobsters of the genus *Thenus* (Crustacea: Decapoda: Scyllaridae), with descriptions of three new species. Zootaxa. 2007;**1429**:1-38

[5] Yang CH, Bracken-Grissom H, Kim D, Crandall KA, Chan TY. Phylogenetic relationships, character evolution, and taxonomic implications within the slipper lobsters (Crustacea: Decapoda: Scyllaridae). Molecular Phylogenetics and Evolution. 2012;**62**(1):237-250

[6] Davis KE, Hesketh TW, Delmer C, Wills MA. Towards a supertree of Arthropoda: A species-level supertree of the spiny, slipper and coral lobsters (Decapoda: Achelata). PLoS ONE. 2015;**10**(10):e0140110. DOI: 10.1371/journal.pone.0140110

[7] Spanier E, Lavalli KL. Slipper lobster fisheries—Present status and future perspectives. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster, Crustacean Issues 17. Vol. 2007. New York: CRC Press, Francis & Taylor Group; 2007. pp. 377-391

[8] Vijayakumaran M, Radhakrishnan EV. Slipper lobsters. In: Fotedar R, Phillips B, editors. Recent Advances and New Species in Aquaculture. Oxford, UK: Wiley-Blackwell; 2011. pp. 85-102

[9] Özcan T, Ateş AS, Bakir K, Katağan T. Commercial crustaceans on the Levantine Sea coast of Turkey. In: Turan C, Salihoğlu B, Özgür Özbek E, Öztürk B, editors. The Turkish Part of the Mediterranean Sea: Marine Biodiversity, Fisheries, Conservation and Governance. Turkish Marine Research Foundation (TUDAV), Publication No: 43; Istanbul, Turkey; 2016. 2016. pp. 392-406. Available from: http://tudav.org/wp-content/uploads/2018/04/MEDITERRANEAN_SEA_2016.pdf

[10] Alborés I, García-Soler C, Fernández L. Reproductive biology of the slipper lobster *Scyllarus arctus* in Galicia (NW Spain): Implications for fisheries management. Fisheries Research. 2019;**212**:1-11

[11] Fujii Y, Fujiwara T, Koide Y, Hasan I, Sugawara S, Rajia S, et al. Internalization of a novel, huge lectin from *Ibacus novemdentatus* (slipper lobster) induces apoptosis of mammalian cancer cells. Glycoconjugate Journal. 2017;**34**(1):85-94

[12] Patek SN, Feldmann RM, Porter M, Tshudy D. Phylogeny and evolution of lobsters. In: Phillips BF, editor. Lobsters: Biology, Management, Aquaculture and Fisheries. Oxford: Blackwell Publishing; 2006. pp. 113-145

[13] Bracken-Grissom HD, Ahyong ST, Wilkinson RD, Feldmann RM, Schweitzer CE, Breinholt JW, et al. The emergences of the lobsters: Phylogenetic relationships, morphological evolution and divergence time comparisons of an ancient group (Decapoda: Achelata, Astacidea, Glypheidea, Polychelidae). Systematic Biology. 2014;**63**:457-479

[14] Schweitzer CE, Feldmann RM. Lobster (Decapoda) diversity and evolutionary patterns through time. Journal of Crustacean Biology. 2014;**34**(6):820-847

[15] Nyborg T, Garassino A. A new genus of slipper lobster (Crustacea: Decapoda: Scyllaridae) from the Eocene of California and Oregon (USA). Neues Jahrbuch für Geologie und Paläontologie (Abhandlungen). 2017;**283**(3):309-316

[16] Audo D. First occurrence of Ibacinae (Eucrustacea: Decapoda: Scyllaridae) from the Eocene of Pakistan. Journal of Systematic Palaeontology. 2019;**17**(6):533-538

[17] Chan TY. Annotated checklist of the world's marine lobsters (Crustacea: Decapoda: Astacidea, Glypheidea, Achelata, Polychelida). The Raffles Bulletin of Zoology. 2010;**23**:153-181

[18] Lavalli KL, Spanier E. Introduction to the biology and fisheries of slipper lobsters. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster. Crustacean Issues 17. New York: CRC Press, Taylor & Francis Group; 2007. pp. 3-21

[19] Holthuis LB. A revision of the family Scyllaridae (Crustacea: Decapoda: Macrura). 1. Subfamily Ibacinae. Zoologische Verhandelingen. 1985;**218**:1-130

[20] Holthuis LB. The Indo-Pacific scyllarine lobsters (Crustacea, Decapoda, Scyllaridae). Zoosystema. 2002;**24**(3):499-683

[21] Wolfe JM, Breinholt JW, Crandall KA, Lemmon AR, Lemmon EM, Timm LE, et al. A phylogenomic framework, evolutionary timeline and genomic resources for comparative studies of decapod crustaceans. Proceedings of the Royal Society B. 2019;**286**(1901):20190079

[22] Genis-Armero R, Guerao G, Abelló P, Ignacio González-Gordillo J, Cuesta JA, Corbari L, et al. Possible amphi-Atlantic dispersal of *Scyllarus* lobsters (Crustacea: Scyllaridae): Molecular and larval evidence. Zootaxa. 2017;**4306**(3):325-338

[23] Robertson RB. The larval development of some western Atlantic lobsters of the family Scyllaridae [thesis]. Coral Gables, Florida: University of Miami; 1968

[24] Robertson PB. The early larval development of the scyllarid lobster *Scyllarides aequinoctialis* (Lund) in the laboratory, with a revision of the larval characters of the genus. Deep-Sea Research. 1969;**16**:557-586

[25] Crosnier A. Naupliosoma, phyllosomes et pseudibacus de *Scyllarides herklotsi* (Herklots) (Crustacea, Decapoda, Scyllaridae) recoltes par l'ombango dans le sud du Golfe de Guinee. Cahiers O.R.S.T.O.M. Oceanographie. 1972;**10**:139-149

[26] Martins HR. Biological studies of the exploited stock of the Mediterranean locust lobster *Scyllarides latus* (Latreille, 1803) (Decapoda: Scyllaridae) in the Azores. Journal of Crustacean Biology. 1985;**5**(2):294-305

[27] Lesser JHR. Identification of early larvae of New Zealand spiny and shovel-nosed lobsters (Decapoda, Palinuridae and Scyllaridae). Crustaceana. 1974;**27**:259-277

[28] Harada E. Notes on the naupliosoma and newly hatched phyllosoma of *Ibacus ciliatus* (Von Siebold). Publications of the Seto Marine Biological Laboratory. 1958;**7**:173-179

[29] Booth JD, Webber WR, Sekiguchi H, Coutures E. Diverse larval recruitment strategies within the Scyllaridae. New Zealand Journal of Marine and Freshwater Research. 2005;**39**:581-592

[30] Sekiguchi H, Booth JD, Webber WR. Early life histories of slipper lobsters. In: Lavalli KL, Spanier E,

editors. The Biology and Fisheries of the Slipper Lobster. Crustacean Issues 17. New York: CRC Press, Taylor & Francis Group; 2007. pp. 69-90

[31] Lavalli KL, Factor JR. The feeding apparatus. In: Factor JR, editor. The Biology of the Lobster, *Homarus americanus*. New York: Academic Press; 1995. pp. 467-510

[32] Baisre JA. Phyllosoma larvae and the phylogeny of Palinuroidea (Crustacea: Decapoda): A review. Australian Journal of Marine and Freshwater Research. 1994;**45**:925-944

[33] Johnson MW. The palinurid and scyllarid lobster larvae of the tropical eastern Pacific and their distribution as related to the prevailing hydrography. Bulletin of the Scripps Institute of Oceanography, University of California. 1971;**19**:1-36

[34] Johnson MW. The phyllosoma larvae of slipper lobsters from the Hawaiian Islands and adjacent areas (Decapoda, Scyllaridae). Crustaceana. 1971;**20**:77-103

[35] Yeung C, McGowan MF. Differences in inshore-offshore and vertical distribution of phyllosoma larvae of *Panulirus*, *Scyllarus* and *Scyllarides* in the Florida keys in May–June, 1989. Bulletin of Marine Science. 1991;**49**:699-714

[36] Coutures E. Distribution of phyllosoma larvae of Scyllaridae and Palinuridae (Decapoda: Palinuridae) in the south-western lagoon of New Caledonia. Marine and Freshwater Research. 2000;**51**:363-369

[37] Minami H, Inoue N, Sekiguchi H. Vertical distributions of phyllosoma larvae of palinurid and scyllarid lobsters in the western North Pacific. Journal of Oceanography. 2001;**57**:743

[38] Shojima Y. Scyllarid phyllosomas' habit of accompanying the jelly-fish. Bulletin of the Japanese Society of Scientific Fisheries. 1963;**29**:349-353

[39] Thomas LR. Phyllosoma larvae associated with medusae. Nature. 1963;**198**:208

[40] Herrnkind WF, Halusky J, Kanciruk P. A further note on phyllosoma larvae associated with medusae. Bulletin of Marine Science. 1976;**26**:110-112

[41] Ates R, Lindsay DJ, Sekiguchi H. First record of an association between a phyllosoma larva and a prayid siphonophore. Plankton and Benthos Research. 2007;**2**:66-69

[42] Palero F, Guerao G, Clark PF, Abelló P. *Scyllarus arctus* (Crustacea: Decapoda: Scyllaridae) final stage phyllosoma identified by DNA analysis, with morphological description. Journal of the Marine Biological Association of the United Kingdom. 2011;**91**(2):485-492

[43] Wakabayashi K, Yang CH, Shy JY, He CH, Chan TY. Correct identification and redescription of the larval stages and early juveniles of the slipper lobster *Eduarctus martensii* (Pfeffer, 1881) (Decapoda: Scyllaridae). Journal of Crustacean Biology. 2017;**37**(2):204-219

[44] Mikami S, Takashima F. Development of the proventriculus in larvae of the slipper lobster, *Ibacus ciliatus* (Decapoda: Scyllaridae). Aquaculture. 1993;**116**:199-217

[45] Mikami S, Greenwood JG, Takashima F. Functional morphology and cytology of the phyllosomal digestive system *Ibacus ciliatus* and *Panulirus japonicus* (Decapoda, Scyllaridae and Palinuridae). Crustaceana. 1994;**67**:212-225

[46] Suzuki N, Murakami K, Takeyama H, Chow S. Molecular attempt to identify prey organisms of lobster phyllosoma larvae. Fishery Science. 2006;**72**:342-349

[47] Gopakumar G, Madhu K, Jayashankar R, Madhu R, Kizhakudan JK, Jose J, et al. Live feed research for larviculture of marine finfish and shellfish. Marine Fisheries Information Service India, T&E Series. 2008;**197**:1-6

[48] Wakabayashi K, Sato R, Ishii H, Akiba T, Nogata Y, Tanaka Y. Culture of phyllosomas of *Ibacus novemdentatus* (Decapoda: Scyllaridae) in a closed recirculating system using jellyfish as food. Aquaculture. 2012;**330**:162-166

[49] Wakabayashi K, Nagai S, Tanaka Y. The complete larval development of *Ibacus ciliatus* from hatching to the nisto and juvenile stages using jellyfish as the sole diet. Aquaculture. 2016;**450**:102-107

[50] Wakabayashi K, Sato H, Yoshie-Stark Y, Ogushi M, Tanaka Y. Differences in the biochemical compositions of two dietary jellyfish species and their effects on the growth and survival of *Ibacus novemdentatus* phyllosomas. Aquaculture Nutrition. 2016;**22**(1):25-33

[51] Wakabayashi K, Phillips BF. Morphological descriptions of laboratory reared larvae and post-larvae of the Australian shovel-nosed lobster *Thenus australiensis* Burton & Davie, 2007 (Decapoda, Scyllaridae). Crustaceana. 2016;**89**(1):97-117

[52] Kamio M, Wakabayashi K, Nagai H, Tanaka Y. Phyllosomas of smooth fan lobsters (*Ibacus novemdentatus*) encase jellyfish cnidae in peritrophic membranes in their feces. Plankton and Benthos Research. 2016;**11**(3):100. DOI: 10.3800/pbr.11.100

[53] Wakabayashi K, Sato R, Hirai A, Ishii H, Akiba T, Tanaka Y. Predation by the phyllosoma larva of *Ibacus novemdentatus* on various kinds of venomous jellyfish. The Biological Bulletin. 2012;**222**(1):1-5

[54] Kamio M, Furukawa D, Wakabayashi K, Hiei K, Yano H, Sato H, et al. Grooming behavior by elongated third maxillipeds of phyllosoma larvae of the smooth fan lobster riding on jellyfishes. Journal of Experimental Marine Biology and Ecology. 2015;**463**:115-124

[55] Lyons WG. Scyllarid lobsters (Crustacea, Decapoda). Florida Marine Research Laboratory, Memoirs of the Hourglass Cruises. 1970;**1**(4):1-74

[56] Webber WR, Booth JD. Larval stages, developmental ecology, and distribution of *Scyllarus* sp. Z (probably *Scyllarus aoteanus* Powell, 1949) (Decapoda: Scyllaridae). New Zealand Journal of Marine and Freshwater Research. 2001;**35**(5):1025-1056

[57] Johnston D. Feeding morphology and digestive system of slipper lobsters. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster. New York: CRC Press; 2007. pp. 111-132

[58] Williamson DI. Names of larvae in the Decapoda and Euphausiacea. Crustaceana. 1969;**16**:210-213

[59] Kizhakudan JK. Culture potential of the *Thenus orientalis* sand lobster (Lund). In: Kurup BM, Ravindran K, editors. Sustain Fish. Cochin, India: School of Industrial Fisheries, Cochin University of Science & Technology; 2006. pp. 256-263

[60] Atkinson JM, Boustead NC. The complete larval development of the scyllarid lobster *Ibacus alticrenatus* bate, 1888 in New Zealand waters. Crustaceana. 1982;**42**:275-287

[61] Stewart J, Kennelly SJ. Fecundity and egg-size of the Balmain bug *Ibacus peronii* (Leach, 1815) (Decapoda, Scyllaridae) off the east coast of Australia. Crustaceana. 1997;**70**:191-197

[62] Spanier E, Lavalli KL. *Scyllarides* spp. In: Phillips BF, editor. Lobsters: Biology Management, Aquaculture and Fisheries Part 2: Lobsters of Commercial Importance. Oxford, UK: Blackwell; 2006. pp. 462-496

[63] Spanier E, Lavalli KL. First record of an early benthic juvenile likely to be that of the Mediterranean slipper lobster, *Scyllarides latu*s (Latreille, 1802). Crustaceana. 2013;**86**(3):259-267

[64] Anastasopoulou A, Mytilineou C, Smith CJ, Papadopoulou KN. Crustacean prey in the diet of fishes from deep waters of the Eastern Ionian Sea. Journal of the Marine Biological Association of the United Kingdom. 2019;**99**(1):259-267

[65] Hearn A. Evaluación de las poblaciones de langostas en la Reserva Marina de Galápagos. Informe Final 2002-2004. Fundación Charles Darwin y Dirección Parque Nacional Galápagos, Santa Cruz, Galápagos, Ecuador; 2004

[66] Hearn A, Toral-Granda V, Martinez C, Reck G. Biology and fishery of the Galápagos slipper lobster. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster. Crustacean Issues 17. New York: CRC Press (Taylor & Francis Group); 2007. pp. 287-308

[67] Rudloe A. Preliminary studies of the mariculture potential of the slipper lobster, *Scyllarides nodifer*. Aquaculture. 1983;**34**:165-169

[68] Haddy JA, Stewart J, Graham KJ. Fishery and biology of commercially exploited Australian fan lobsters (*Ibacus* spp.). In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster. Crustacean Issues 17. New York: CRC Press, Taylor & Francis Group; 2007. pp. 359-373

[69] Mikami S, Kuballa AV. Larvae and larval rearing of scyllarids. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster. Crustacean Issues 17. New York: CRC Press; 2007. pp. 91-110

[70] Bianchini M, Bono G, Ragonese S. Long-term recaptures and growth of slipper lobsters, *Scyllarides latus*, in the strait of Sicily (Mediterranean Sea). Crustaceana. 2001;**74**(7):673-680

[71] Chessa LA, Pais A, Serra S. Behavioural observations on slipper lobster *Scyllarides latus* (Latreille 1803) (Decapoda, Scyllaridae) reared in laboratory. In: Proceedings of the 6th Colloquium Crustacea Decapoda Mediterranea, Florence; 12-15 September 1996. pp. 25-26

[72] Spanier E, Lavalli KL. Natural history of *Scyllarides latus* (Crustacea: Decapoda): A review of the contemporary biological knowledge of the Mediterranean slipper lobster. Journal of Natural History. 1998;**32**(6):1769-1786

[73] Bianchini ML, Ragonese S. Growth of slipper lobsters of the genus *Scyllarides*. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster. Crustacean Issues 17. New York: CRC Press, Taylor & Francis Group; 2007. pp. 199-220

[74] Almog-Shtayer G. Behavioural-ecological aspects of Mediterranean lobsters in the past and of the slipper lobster, *Scyllarides latus*, in the present [thesis]. Israel: University of Haifa; 1988

[75] DeMartini EE, Williams HA. Fecundity and egg size of *Scyllarides squammosus* (Decapoda: Scyllaridae) at Mare Reef, Northwestern Hawaiian Islands. Journal of Crustacean Biology. 2001;**21**:891-896

[76] Lima FA, Martinelli-Lemos JM, Silva KC, Klautau AG, Cintra IH. Population structure and fecundity of *Scyllarides delfosi* Holthuis,

1960 (Scyllaridae) on the Amazon continental shelf. Crustaceana. 2018;**91**(9):1027-1103

[77] Almeida Duarte LFD, Severino-Rodrigues E, Pinheiro MA, Gasalla MA. Slipper lobsters (Scyllaridae) off the southeastern coast of Brazil: Relative growth, population structure, and reproductive biology. Fishery Bulletin. 2015;**113**:55-68

[78] Bianchini ML, Ragonese S. *In ovo* embryonic development of the Mediterranean slipper lobster, *Scyllarides latus*. The Lobster Newsletter. 2003;**16**:10-12

[79] Spanier E, Tom M, Pisanty S, Almog G. Seasonality and shelter selection by the slipper lobster *Scyllarides latus* in the southeastern Mediterranean. Marine Ecology Progress Series. 1988;**42**:247-255

[80] Stewart J, Kennelly SJ. Growth of the scyllarid lobsters *Ibacus peronii* and *I. chacei*. Marine Biology. 2000;**136**:921-930

[81] Sharp WC, Hunt JH, Teehan WH. Observations on the ecology of *Scyllarides aequinoctialis, Scyllarides nodifer*, and *Parribacus antarcticus* and a description of the Florida scyllarid lobster fishery. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster. Crustacean Issues 17. New York: CRC Press, Taylor & Francis Group; 2007. pp. 231-242

[82] Kagwade PV, Kabli LM. Age and growth of the sand lobster *Thenus orientalis* (Lund) from Bombay waters. Indian Journal of Fisheries. 1996;**43**(3):241-247

[83] Jones CM. Biology and fishery of the bay lobster, *Thenus* spp. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster. Crustacean Issues 17. New York: CRC Press; 2007. pp. 325-358

[84] Radhakrishnan EV, Manisseri MK, Deshmukh VD. Biology and fishery of the slipper lobster, *Thenus orientalis,* in India. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster. Crustacean Issues 17. New York: CRC Press, Taylor & Francis Group; 2007. pp. 309-324

[85] Subramanian VT. Fishery of sand lobster *Thenus orientalis* (Lund) along Chennai coast. Indian Journal of Fisheries. 2004;**51**(1):111-115

[86] Faria J, Froufe E, Tuya F, Alexandrino P, Pérez-Losada M. Panmixia in the endangered slipper lobster *Scyllarides latus* from the northeastern Atlantic and western Mediterranean. Journal of Crustacean Biology. 2013;**33**(4):557-566

[87] Lerosey-Aubril R, Meyer R. The sensory dorsal organs of crustaceans. Biological Reviews. 2013;**88**(2):406-426

[88] Johnston DJ, Alexander CG. Functional morphology of the mouthparts and alimentary tract of the slipper lobster *Thenus orientalis* (Decapoda: Scyllaridae). Marine and Freshwater Research. 1999;**50**:213-223

[89] Suthers IM, Anderson DT. Functional morphology of mouthparts and gastric mill of *Ibacus peronii* (leach) (Palinura: Scyllaridae). Australian Journal of Maine and Freshwater Research. 1981;**32**:931-944

[90] Lau CJ. Feeding behavior of the Hawaiian slipper lobster, *Scyllarides squammosus*, with review of decapod crustacean feeding tactics on molluscan prey. Bulletin of Marine Science. 1987;**41**:378-391

[91] Spanier E. Mollusca as food for the slipper lobster *Scyllarides latus* in the coastal waters of Israel. Levant. 1987;**68**:713-716

[92] Lavalli KL, Malcom CN, Goldstein JS. Description of pereiopod setae

of scyllarid lobsters, *Scyllarides aequinoctialis, Scyllarides latus*, and *Scyllarides nodifer*, with observations on the feeding during consumption of bivalves and gastropods. Bulletin of Marine Science. 2018;**94**(3):571-601

[93] Martínez CE. Ecología trófica de *Panulirus gracilis*, *P. penicillatus* y *Scyllarides astori* (Decapoda, Palinura) en sitios de pesca de langosta en las islas Galápagos [thesis]. Ecuador: Universidad del Azuay; 2000. 102 pp

[94] Ogren LH. Concealment behaviour of the Spanish lobster, *Scyllarides nodifer* (Stimpson), with observations on its diel activity. Northeast Gulf Science. 1977;**1**(2):115-116

[95] Barr L. Some aspects of the life history, ecology and behaviour of the lobsters of the Galápagos Islands. Stanford Oceanographic Expedition. 1968;**17**:254-262

[96] Martínez CE, Toral V, Edgar G. Langostino. In: Danulat E, Edgar GJ, editors. Reserva Marina de Galápagos, Línea Base de la Biodiversidad. Fundación Charles Darwin y Servicio Parque Nacional Galápagos; Santa Cruz, Galápagos, Ecuador; 2002. pp. 216-232

[97] Goldstein JS, Spanier E. In the heat of the moment: Effects of elevated temperature on seasonal movements in slipper lobsters (*Scyllarides latus*) in the eastern Mediterranean. In prep.

[98] Lavalli KL, Spanier E. Does gregariousness function as an antipredator mechanism in the Mediterranean slipper lobster, *Scyllarides latus*? Marine and Freshwater Research. 2001;**52**:1133-1143

[99] Moe MA Jr. Lobsters: Florida, Bahamas, the Caribbean. Plantation, FL: Green Turtle Publications; 1991. 510 pp

[100] Hardwick CW Jr, Cline CB. Reproductive status, sex ratios and morphometrics of the slipper lobster *Scyllarides nodifer* (Stimpson) (Decapoda: Scyllaridae) in the Northeastern Gulf of Mexico. Northeast Gulf Science. 1990;**11**(2):131-136

[101] Faulkes Z. Digging mechanisms and substrate preferences of shovel nosed lobsters, *Ibacus peroni* (Decapoda: Scyllaridae). Journal of Crustacean Biology. 2006;**26**(1):69-72

[102] Spanier E, Weihs D, Almog-Shtayer G. Swimming of the Mediterranean slipper lobster. Journal of Experimental Marine Biology and Ecology. 1991;**145**:15-31

[103] Spanier E, Almog-Shtayer G. Shelter preferences in the Mediterranean slipper lobster: Effects of physical properties. Journal of Experimental Marine Biology and Ecology. 1992;**164**:103-116

[104] Spanier E, Almog-Shtayer G, Fiedler U. The Mediterranean slipper lobster *Scyllarides latus*: The known and the unknown. Bios. 1993;**1**(1):49-58

[105] Barshaw DE, Spanier E. The undiscovered lobster—A first look at the social behaviour of the Mediterranean slipper lobster, *Scyllarides latus* Decapoda. Scyllaridae. Crustaceana. 1994;**67**(2):187-197

[106] Barshaw DE, Spanier E. Anti-predator behaviours of the Mediterranean slipper lobster *Scyllarides latus*. Bulletin of Marine Science. 1994;**55**(2):375-382

[107] Barshaw DE, Lavalli KL, Spanier E. Is offence the best defence: The response of three morphological types of lobsters to predation. Marine Ecology Progress Series. 2003;**256**:171-182

[108] Lavalli KL, Spanier E, Grasso F. Behavior and sensory biology of slipper lobsters. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the

Slipper Lobster. Crustacean Issues 17. New York: CRC Press, Taylor & Francis Group; 2007. pp. 133-181

[109] Zimmer-Faust RK, Spanier E. Gregariousness and sociality in spiny lobsters: Implications for den habitations. Journal of Experimental Marine Biology and Ecology. 1987;**105**:57-71

[110] Spanier E, Zimmer-Faust RK. Some physical properties of shelter that influence den preference in spiny lobsters. Journal of Experimental Marine Biology and Ecology. 1988;**122**:137-149

[111] Lozano-Alvarez E, Spanier E. Behavior and growth of captive sub-adults spiny lobsters, *Panulirus argus,* under the risk of predation. Marine and Freshwater Research. 1997;**48**(8):707-713

[112] Herrnkind WF, Childress MJ, Lavalli KL. Defense coordination and other benefits among exposed spiny lobsters: Inferences from mass migratory and mesocosm studies of group size and behavior. Marine and Freshwater Research. 2001;**52**:1133-1143

[113] Hamilton WD. Geometry for the selfish herd. Journal of Theoretical Biology. 1971;**31**:294-311

[114] Horne FR, Tarsitano SF. The mineralization and biomechanics of the exoskeleton. In: Lavalli KL, Spanier E, editors. The Biology and Fisheries of the Slipper Lobster. Crustacean Issues 17. New York: CRC Press, Taylor & Francis Group; 2007. pp. 183-198

[115] Tarsitano SF, Lavalli KL, Horne F, Spanier E. The constructional properties of the exoskeleton of homarid, scyllarid, and palinurid lobsters. Hydrobiologia. 2006;**557**:9-20

[116] Smale MJ, Goosen AJJ. Reproduction and feeding of spotted gully shark, *Triakis megalopterus*, off the Eastern Cape, South Africa. Fishery Bulletin. 1999;**97**:987-998

[117] Barreiros JP, Santos RS. Notes on the food habits and predatory behaviour of the dusky grouper, *Epinephelus marginatus* (Lowe, 1834) (Pisces: Serranidae) in the Azores. Arquipelago Life and Marine Sciences. 1998;**16A**:29-35

[118] Mizrahi M, Chapman JK, Gough CLA, Humber F, Anderson LG. Management implications of the influence of biological variability of invasive lionfish diet in Belize. Management. 2017;**8**(1):61-70

[119] Oliveira G, Freire AS, Bertuol PRK. Reproductive biology of the slipper lobster *Scyllarides deceptor* (Decapoda: Scyllaridae) along the southern Brazilian coast. Journal of the Marine Biological Association of the United Kingdom. 2008;**88**:1433-1440

[120] DeMartini EE, McCracken ML, Moffitt RB, Wetherall JA. Relative pleopod length as an indicator of size at sexual maturity in slipper (*Scyllarides squammosus*) and spiny Hawaiian (*Panulirus marginatus*) lobsters. Fishery Bulletin. 2005;**103**:23-33

[121] Kizhakudan JK, Thirumilu P, Rajapackiam S, Manibal C. Captive breeding and seed production of scyllarid lobsters—Opening new vistas in crustacean aquaculture. Marine Fisheries Information Service India, T & E Series. 2004;**181**:1-4

[122] Goldstein JS, Dubofsky EA, Spanier E. Into a rhythm: Diel activity patterns and behaviour in Mediterranean slipper lobsters, *Scyllarides latus*. ICES Journal of Marine Science. 2015;**72**(Suppl. 1):i147-i154

[123] O'Malley JM, Walsh WA. Annual and long-term movement patterns of spiny lobster, *Panulirus marginatus*, and

slipper lobster, *Scyllarides squammosus*, in the Northwestern Hawaiian islands. Bulletin of Marine Science. 2013;**89**(2):529-549

[124] Stewart J, Kennelly SJ. Contrasting movements of two exploited Scyllarid lobsters of the genus *Ibacus* off the east coast of Australia. Fisheries Research. 1998;**2-3**:127-132. DOI: 10.1016/S0165-7836(98)00104-0

[125] Neil DM, Ansel AD. The orientation of tail-flip escape swimming in decapod and mysid crustaceans. Journal of the Marine Biological Association of the United Kingdom. 1995;**75**:55-70

[126] Ritz DA, Jacklyn PM. Believe it or not—Bugs fly through water. Australian Fisheries. 1985;**44**:35-37

[127] Jacklyn PM, Ritz DA. Hydrodynamics of swimming in scyllarid lobsters. Journal of Experimental Marine Biology and Ecology. 1986;**101**:85-89

[128] Faulkes Z. Loss of escape responses and giant neurons in the tailflipping circuits of slipper lobsters, *Ibacus* spp. (Decapoda, Palinura, Scyllaridae). Arthropod Structure and Development. 2004;**33**:113-123

[129] Jones C. The biology and behaviour of bay lobsters, *Thenus* spp. (Decapoda: Scyllaridae) in Northern Queensland, Australia [thesis]. Brisbane, Australia: University of Queensland; 1988

[130] Spanier E, Weihs D. Why do shovel-nosed (slipper) lobsters have shovels? The Lobster Newsletter. 1992;**5**(1):8-9

[131] Spanier E, Weihs D. Hydrodynamic aspects of locomotion in the Mediterranean slipper lobster, *Scyllarides latus*. In: Proceedings of the 7th International Conference and Workshop on Lobster Biology and Management; 8-13 February 2004; Tasmania, Australia. p. 61

[132] Bianchini ML, Raisa PF. Valutazione della fattibilità e potenzialità del ripoplamento attivo per la magnosa, Scyllarides latus (*Crostacei Decapodi*). Final report to MiRAAF (Pesca Marittima); 1997; 1

[133] Newell IM. A parasitic species of *Copidognathus* (Acari: Halacaridae). Proceedings of the Hawaiin Entomollogical Society. 1956;**16**(1):122-125

[134] Perry LT. Relationships between culture conditions and moult death syndrome (MDS) in larval development of the bay lobsters *Thenus orientalis* (Lund, 1793) and *Thenus indicus* Leach, 1815 (Decapoda: Scyllaridae) [thesis]. School of Biological Sciences, University of Queensland; 2001

[135] Wakabayashi K, Otake S, Tanaka Y, Nagasawa K. *Choniomyzon inflatus* n. sp. (Crustacea: Copepoda: Nicothoidae) associated with *Ibacus novemdentatus* (Crustacea: Decapoda: Scyllaridae) from Japanese waters. Systematic Parasitology. 2013;**84**(2):157-165

[136] WWF. 2015. Living Blue Planet Report. Species, habitats and human well-being. In: Tanzer J, Phua C, Lawrence A, Gonzales A, Roxburgh T, Gamblin P, editors. WWF, Gland, Switzerland. Available from: https://www.wwf.de/fileadmin/fm-wwf/Publikationen-PDF/Living-Blue-Planet-Report-2015.pdf

[137] Gillespie KM, Vincent AC. Tropical invertebrate response to marine reserves varies with protection duration, habitat type, and exploitation history. Aquatic Conservation: Marine and Freshwater Ecosystems. 2019;**29**(3):511-520

[138] Miller E, Spanier E, Yahel R, Diamant R. Do marine nature reserves enhance the conservation

of the Mediterranean slipper lobster (*Scyllarides latus*)?—Preliminary results. In: Proceedings of the 2nd Israeli Conference for Conservation Science—A Sustainable Future for Humans and Nature; 16-17 April 2019; Technion, Haifa. p. 24

[139] Groeneveld JC, Kirkman SP, Boucher M, Yemane D. From biomass mining to sustainable fishing—Using abundance and size to define a spatial management framework for deep-water lobster. African Journal of Marine Science. 2012;**34**(4):547-557

[140] Schultz JK, O'Malley JM, Kehn EE, Polovina JJ, Parrish FA, Kosaki RK. Tempering expectations of recovery for previously exploited populations in a fully protected marine reserve. Journal of Marine Biology. 2011;**2011**. DOI: 10.1155/2011/749131

[141] Boavida-Portugal J, Rosa R, Calado R, Pinto M, Boavida-Portugal I, Araújo MB, et al. Climate change impacts on the distribution of coastal lobsters. Marine Biology. 2018;**165**(12):186

Chapter 4

Management of the Interaction and Cannibalism of Postlarvae and Adults of the Freshwater Shrimp *Cryphiops caementarius* (Molina, 1782)

Walter Reyes A.

Abstract

Cryphiops caementarius shrimp inhabits the rivers of the western slope of the Andes of Peru and Chile. But the greatest population densities found in the rivers of Arequipa (Peru) have social, economic, commercial, and gastronomic importance. Researches on this species of shrimp date from 1950. The males of *C. caementarius* are aggressive by having one of the most developed chelipeds, causing greater interaction and cannibalism. To reduce the interaction of the species, it has been used two culture systems. For postlarvae, using brackish water can maintain high survival (>85%), but only in initial culture which lasts for 50 days. For the fattening of adult males, culturing in separate containers conditioned in various levels improves the survival (87–100%) and yield (1.0 kg m^{-2}), and with this system, the culture is also performed with tilapia. It is still required to demonstrate the technical and economic feasibility of fattening male shrimp in individual containers within seminatural ponds.

Keywords: freshwater shrimp, cannibalism, interaction, culture systems

1. Introduction

The Palaemonid shrimps that inhabit the rivers of the western slope of the Andes are represented by 12 species, three of which correspond to the genus *Palaemon*, eight to *Macrobrachium,* and one to *Cryphiops* [1]. Of these, *Cryphiops caementarius* (Molina, 1782) inhabits the rivers of the coast of Peru and Chile. However, only in Peru, it has social, economic, and commercial importance since it is extracted from the Pativilca River in Lima to the Tambo River in Arequipa, where there is high population density [2], which, in 2016, was captured as 1112.9 t [3]. In addition, the species has culinary importance whose potential markets are restaurants in the regions of Lima and Arequipa in Peru [4]. *C. caementarius* is also distributed until Valparaiso in Chile [5], although with less commercial importance due to the low population densities and because it is a vulnerable species in the northern region and in danger of extinction in the Metropolitan Region of Chile [6].

Other species of *Cryphiops* inhabit caves in the state of Chiapas in Mexico, as *C.* (*Bithynops*) *luscus* and *C.* (*Bithynops*) *perspicax* [7] and *C. (Bithynops) villalobosi* inhabits rivers and streams [8]. In Brazil, *C. brasiliensis* inhabit in a river of the Federal District [9]. All these species are small in size, whose populations are not attractive to trade.

Researches related to shrimp *C. caementarius* date from 1950, and the generated interest is in order to establish commercial cultivation. However, the culturing is affected as the strong interaction given the size and thicker of the second pair of pereiopods that is a sign that the species is aggressive, and for the cannibalism that happens between congeners. These limitations affect the growth and yield of shrimp.

The purpose of this chapter was to review progress in research with that of the freshwater shrimp *C. caementarius*, related to alternative solutions to the problems of the management of the interaction and cannibalism of postlarvae and adults.

2. Interaction

In decapod crustaceans, there are those who are very aggressive as portunids crabs (*Scylla*, *Callinectes*, and *Portunus*), king crabs (*Lithodes* and *paralithodes*), followed by chelated lobsters (*Homarus* and *Nephrops*) and spiny lobsters (*Panulirus* and *Jasus*), and also, those who are less aggressive as crayfish (*Procambarus*, *Cherax*, *Pacifastacus*, and *Astacus*) and penaeids (*Litopenaeus*) and are less cannibals [10]. Therefore, the aggressiveness between congeners depends on the species.

In the territorialist decapod crustaceans, the second pair of pereopods (chelipeds) is long and thick and also those are used for attack and defense, for agonistic interaction and for courtship and mating [11]. Males of *C. caementarius* have one of the most developed chelipeds (**Figure 1**), either the right or the left. In females, the chelipeds are of similar size. This morphological feature of the chelipeds makes males an aggressive species whose interaction and cannibalism are observed in aquariums, tanks, and ponds [12], but this behavior has not been assessed yet. In *Callinectes arcuatus* and *C. bellicosus*, the chelipeds are dimorphic, and generally, the right cheliped is the largest and the thicker that permit to consume mollusks and crustaceans [13].

The interaction of male *C. caementarius* is greater than that of females, because of this situation, males always show serious injuries in the cephalothorax, abdomen, and chelipeds, although it is common to observe shrimps without chelipeds. In juvenile *C. caementarius*, interaction and increasing stocking density cause high metabolic rate (87–91%) that affects the growth in weight [14] and probably the physiological state of the animal. In *Macrobrachium rosenbergii*, the chelipeds of older males are larger and thicker with which they access easily to food and shelter, in addition to giving them greater ability to combat due to the visualization of the

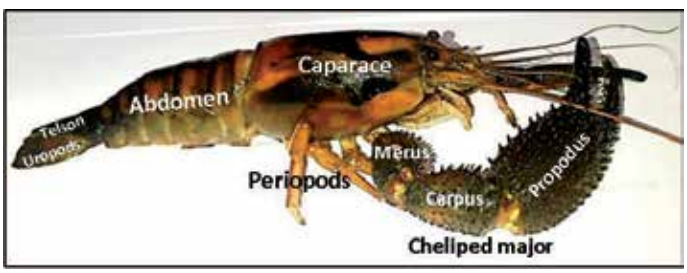

Figure 1.
Body parts and appendages of the freshwater shrimp C. caementarius.

opponent [15, 16]. This explains why the most affected parts of the crustaceans are the pereopods, pleopods, antennae, antennules, and uropods [17].

3. Cannibalism

All decapod crustaceans use chelipeds for interaction, access to food, shelter, and mating, resulting in energy expenditure during the fight and can reach the autotomy of appendages and even cannibalism. Cannibalism, defined as intraspecific predation, is a behavior established in a wide variety of animals [18] and is considered as the process of killing and eating an individual of the same species [19], whether it consumes all or part of it. In *C. caementarius*, cannibalism is often a response to captivity, lack of shelter, ecdysis, increased density, lack of food, and poor water quality, and it is probably a natural behavior.

The molting fluid accumulated between the old cuticle and epidermis is the product of degradation of the old cuticle [20], which is released with ecdysis and acts as a chemical stimulant [21]. In the Hermit crabs, *Clibanarius digueti* and *Paguristes perrieri*, the odor of the injured animals of the same species as well as other species is a feeding signal [22]. Similarly, the odor released during autotomy to escape a predatory aggression influences agonistic behavior in decapod crustaceans [10]. The *C. caementarius* adults who are close to the ecdysis (premuda D3 and D4) during ecdysis (E) or after ecdysis (postmoult A) are more prone to cannibalism, mainly after the ecdysis, where the soft exoskeleton, which takes time to harden, makes movement and defense difficult for himself. The cannibalism of C. caementarius starts from Zoea 8 and increases as they grow [23, 24]. These observations indicate that cannibalism may have a genetic component, at least in the species, as suggested in other cannibalistic species [19].

In *M. rosenbergii*, interaction and cannibalism by molt is attenuated with the use of shelters [25, 26], artificial substrates [27, 28], and by increasing tryptophan in the diet [29], with which high survival is maintained and growth is improved. Further research is needed to evaluate these culturing systems in *C. caementarius*.

4. Cultivation of postlarvae in brackish water

The crustacean's cultivation comprises producing postlarvae in hatchery, the postlarvae growth until reaching juvenile stage or condition of preadult and the fattening until reaching commercial weight (>20 g). Postlarvae adapt to environmental conditions during the initial culture, and those who survive are resistant, and have higher growth rate. However, as mentioned, the problem is the interaction and cannibalism that happens throughout the animal's life. Similar advantages are reported during the nursery phase of *M. rosenbergii* postlarvae due to the interaction and cannibalism [30].

To reduce cannibalism of postlarvae during communal cultivation and obtain juveniles with greater weight (200 mg) for stocking in ponds, growing in brackish water should be performed. Recent postlarvae of *C. caementarius* (11 mm total length and 40 mg total weight) have remarkable euryhalinity and achieve greater weight when grown for 50 days in brackish water of 12‰ and with density of 114 PLs m^{-2} [31]. These results demonstrate the physiological efficiency of organisms to accumulate biomass in such salinity conditions, probably because they are in their isosmotic point. In addition, 95% of postlarvae survive in water with salinity of 12‰, 70% live in water of 24‰, and 40% in fresh water. This high survival of postlarvae in brackish water than in fresh water is due to the reduction

of cannibalism probably because the released substances before, during, and after ecdysis are attenuated by ions of the brackish water from the culture medium [31].

Furthermore, the culture of postlarvae *C. caementarius* in brackish water with 12‰ allows increasing the density up to 500 PLs m^{-2} without affecting the growth and survival after 60 days of culture [32]. In juveniles of *M. tenellum* [33] and *M. rosenbergii*, the higher growth and higher survival (>90%) are obtained in water with 10‰ salinity [34]. Under these conditions of salinity and density, it is convenient to use shelters or artificial substrates to enhance the growth of postlarvae.

5. Adult shrimp culturing in individual containers

The main problems of the communal culturing of adult crustaceans are the interaction and cannibalism per molt, which are accentuated as the animals grow and affect the growth and survival, respectively. In the communal culturing of *C. caementarius*, survival decreases to 17% in aquariums [12] and 25% in tanks [24, 35]. In seminatural ponds, it is likely to obtain survivals between 40 and 50%, and even the density is 5 shrimp m^{-2}, due to increased cannibalism. These survival results of the species prevent the establishment of commercial cultivation.

Cultivation in individual containers was first used in lobster *H. americanus* where the container shape (circular, square, and rectangular) does not affect the growth, but the size of these (20–181 cm^2) retards the growth [36]. Larger containers were also used (750 cm^2) [37]. The cultures in individual containers and conditioned at several levels used are *Cherax tenuimanus* [38], *C. quadricarinatus* [39], and *H. americanus* [40, 41]. Although the circular containers can have mesh as used in *H. gammarus* [42]. In any type of culture container, physical interaction of organisms is avoided, improving the growth and survival.

The first cultivation system in individual containers was performed with adult females *C. caementarius* [43] and then with males [12], both in aquariums (**Figure 2**) and fiberglass tanks (**Figure 3**). In this system, the species tolerates cultivation in containers of reduced physical space (133–284 cm^2), not being affected by ovarian maturation, the spawning, the molting period, and the growth and survival during 4–6 months of culture. Moreover, the lower specific density factor k = 16 means that the species requires less space than the other crustaceans [12] obtained. The specific density factor is an indicator when the size of the containers inhibits the species growth, and the k factor is ≤ 22 from *C. quadricarinatus* [39], $k \leq 45$ from *C. tenuimanus* [38], and $k \leq 50$ from *C. destructor* [44]. That is, these species of crustaceans cannot tolerate reduced physical spaces during cultivation in individual containers.

Figure 2.
System culture of C. caementarius in individual containers conditioned in aquariums with water recirculation system and biofilter.

Figure 3.
System culture of C. caementarius in individual containers conditioned in fiberglass tanks with water recirculation system and biofilter.

Individual containers are conditioned in various levels, both aquariums and tanks or seminatural ponds, thus increasing the planting density. In aquariums (0.186 m^2 and effective volume of 55 L), the containers are installed in three levels, but in two columns, making a total of six containers per aquarium equivalent to 32 shrimps m^{-2} (**Figure 2**). In fiberglass tanks (with a bottom area of 0.159 m^2 and an effective volume of 100 L), the containers are installed in five levels, but in three columns, making a total of 15 containers per tank equivalent to 94 shrimps m^{-2} (**Figure 3**). In both cases, the increased production is achieved in large containers (284 cm^2), although no significant differences with those of smaller areas (**Table 1**) are seen. In *C. quadricarinatus*, the culture containers are conditioned in seven levels within 3 m^2 tanks where high yield in containers of 490 cm^2 is achieved [39]. But, as shrimp *C. caementarius* is sold by weight, including the chelipeds, then it is preferable to use the large containers. Shrimp farming in individual containers installed in seminatural ponds has not been investigated, but environmental and productivity pond water conditions could benefit the growth, color of the shrimp, and reduce the feed conversion.

The effective density is the number of surviving organisms at the end of the culture period according to the area of the container. In *C. caementarius*, the effective density of 94 shrimps m^{-2}, obtained by cultivation in individual containers, is considered high, and therefore, cultivation is intensive, which could produce 10.5 t ha^{-1} per period in 4 months (**Table 1**), and get 31.5 t ha^{-1} y^{-1}, which is 10 times higher than that one obtained in semi-intensive monoculture of *M. rosenbergii* reaching 3 t ha^{-1} y^{-1} [45]. However, in *C. quadricarinatus*, stocked in individual containers, the final effective density after 100 days of culture was between 143 and 348

Container		Effective density (shrimp m^{-2})	Final weight (g)	Estimated performance (kg m^{-2})	Estimated production* (t ha^{-1} $period^{-1}$)
Area (cm^2)	Diameter (cm)				
133	13	94.34 ± 0.00	8.09 ± 1.37[a]	0.763 ± 0.129[a]	7.64 ± 1.29[a]
201	16	94.34 ± 0.00	9.99 ± 0.62[ab]	0.941 ± 0.058[a]	9.42 ± 0.59[a]
284	19	81.76 ± 10.89	13.20 ± 1.99[b]	1.049 ± 0.059[a]	10.49 ± 0.59[a]

**Data were estimated for a 4-month period. Letters a and b in superscript in a column indicate that there is a significant difference ($p < 0.05$).*

Table 1.
Estimated production (mean ± standard deviation) of males of C. caementarius cultivated during 4 months in individual containers of different sizes conditioned in five levels inside fiberglass tanks [12].

individuals m^{-2} and the yield between 4 and 8 kg m^{-2} [39]. Future research should establish in detail the conditions of the cultivation system in individual containers, to intensify the cultivation of *C. caementarius*. Parallel to this, a technical-economic study must be made to know the feasibility of shrimp farming in the system.

However, the cultivation of *C. caementarius* in individual containers causes loss of body color and of cephalothoracic appendages (**Figure 4**), but the use of *Capsicum annuum* (250 mg kg^{-1}) [12] and in the diet (300 mg kg^{-1}) improves pigmentation of the body [46]. Consequently, the shrimp diet should contain carotenoid pigments, since crustaceans cannot synthesize carotenoids de novo [47]. Body depigmentation attributed to diet happens in *C. tenuimanus* [38] and *H. gammarus* [40], when they are grown in individual containers.

In the culture of *C. caementarius* in individual containers, the management of food at the commercial level would imply a cost of additional labor that increases the cost of cultivation. It is, therefore, necessary to design a food distribution system as used in *Homarus* sp. [48] and *H. gammarus* [49]. In species of *Scylla*, the most sophisticated designed system to date includes cameras linked to a computer system that regularly scans the cells to see if there are one or two crabs in each container indicating that the crab has made an ecdysis, by the presence of exoskeleton and crab. In addition, this system also includes a sophisticated water recirculation system [50].

In studies with male shrimps *C. caementarius* in individual containers installed in aquaria and tanks with recirculation system with water and biological filters, growth is evaluated by eyestalk ablation [51] per culturing at different water hardness [52] and by different inputs used in the diet such as paprika [46], yeast [53], common salt [54], biological silage [55], and soya lecithin [56]. Survival of >90% are obtained in all these investigations, which demonstrates the effectiveness of the culture system by avoiding the physical interaction and cannibalism of river shrimp. In similar culture conditions, survivals between 71 and 83% in *C. tenuimanus* [38] and 96% in *C. quadricarinatus* [39] are achieved. However, the system requires individual containers to be improved with regard to handling molting, feed system, monitoring of the species, the recirculation system automation, and use in seminatural ponds. In the same way, the individual containers are not only useful for enhancing the growth of *C. caementarius*, but also for the management of female reproduction, and in the case of shrimp males, also for selecting those with the highest rates of specific growth (>1% weight day^{-1}) for the purposes of genetic improvement.

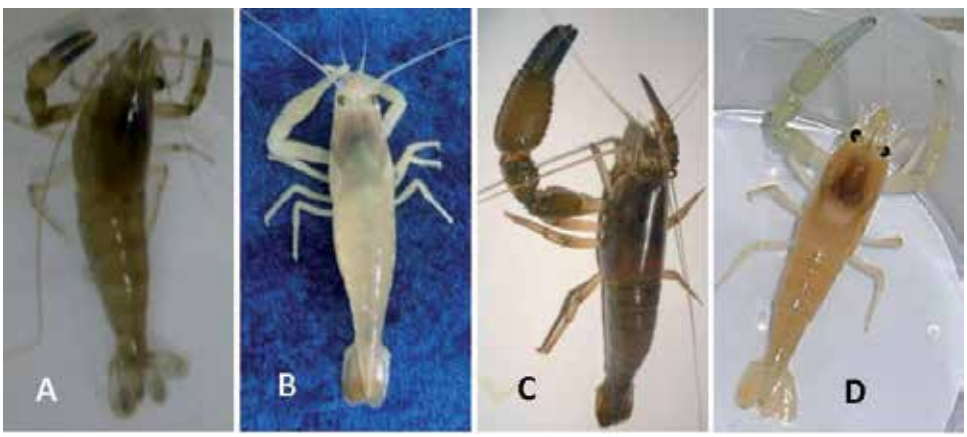

Figure 4.
Color of the C. caementarius body after culturing in individual containers: (A) female of normal color; (B) female depigmented; (C) male of normal color; and (D) male depigmented.

6. Co-culture of shrimp/tilapia

The co-culturing is done out with two species share a common aquatic environment (aquarium, tank, or pond), but whose construction does not allow physical interaction between organisms because they remain separate, and therefore, both species are a major management factor. Instead, in the polyculture, two or three species within the aquatic environment interact constantly competing for space and food, and therefore only one species is the main one.

Combinations of species in a co-culturing allow maximizing the performance of those who are territorial and aggressive. The co-culture of *Oreochromis niloticus* in cages inside ponds with *M. rosenbergii* [57] is well known. Also, it is known to co-culture of *C. caementarius* male shrimp in individual containers inside aquariums with *O. niloticus* fingerlings (**Figure 5**), where tilapia production was estimated at 0.511 kg m^{-3}. The tilapia consumed only food that came out of the shrimp culture container [12]. In other researches, co-culture of *C. caementarius* shrimp with tilapia *O. niloticus* at different densities were performed [58], and co-culturing shrimp with tilapia was performed to evaluate different concentrations of biological silage [55]. In both cases, 100% survival was achieved and production of species is improved. Co-cultivation of shrimp/tilapia mainly generates high nitrates that may be used in the cultivation of vegetables, whose integration would result in an aquaponics system.

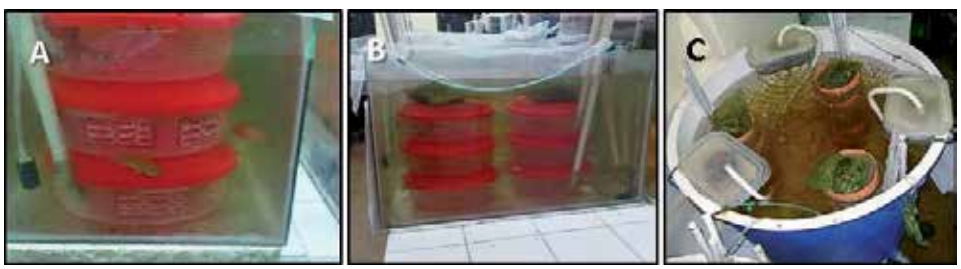

Figure 5.
(A) Co-culture of C. caementarius in individual containers with O. niloticus. (B) System of co-culture in aquarium. (C) Co-culture system in fiberglass tank.

7. Transportation of adult shrimp

The communal transport of male adult shrimps *C. caementarius* for fattening purposes is difficult due to the increased interaction causing injuries, chelipeds loss, shrimp death mainly of those who are about to carry out ecdysis or those who molt during the transport. In addition, the increased mortality depends on the density and transport time; but water temperature is a dominant factor that affects shrimp during transport [59].

The transport of adult *C. caementarius* shrimps in individual containers is performed by using plastic cups where a shrimp (≥4 cm of total length) is introduced into each plastic cup (250 mL). The plastic cup has holes to allow the water flow (**Figure 6A**). Then, all plastic cups are conditioned in plastic containers (45 L) with water of river (**Figure 6B**) and with either continuous or intermittent aeration (**Figure 6C**). The average water temperature is around 20°C. This system allows to transport 77 shrimps (17 shrimps for 10 L) per container for 5 h and with 100% survival [12]. However, the ideal size of transporting cups of live shrimps has not been studied, but as they support very small physical space, it is possible to use smaller plastic cups or to use PVC pipes according to the animal size.

Figure 6.
Transport system of live shrimps of C. caementarius. (A) Plastic cup with a shrimp. (B) Plastic cups put into a plastic container. (C) Aerator system.

On the other hand, the conventional transport of *C. caementarius*, an adult shrimp of 6 cm of total length, is also carried out in 4‰ of water salinity, because with this salinity, the shrimps do not show interaction or cannibalism and all survive in these conditions for 45 days [60]. The *M. rosenbergii* broodstock are transported in containers with brackish water (12‰), with oxygen and at low temperature to reduce metabolism, thus obtaining mortalities <10% [61]. In addition, aerated plastic barrels, or trucks with aerated water tanks, are used [62]. Other techniques such as increasing air humidity, the use of refrigerated sawdust or chips, and purging to reduce nitrogenous waste have been developed to increase the survival during transportation of live specimens of shrimp, prawns, lobsters, and crabs [63].

8. Conclusions

The male shrimps *C. caementarius* are aggressive for having one of the chelipeds more developed, causing greater interaction and cannibalism in any culture system. Female shrimps are less aggressive. To reduce interaction and shrimp cannibalism, two management systems are proposed. For postlarvae, using brackish water (12‰) keeps high survival (>85%) but only in the initial culture which lasts for 50 days. For the fattening of adult males, growing in individual containers conditioned in multiple levels allows high survival (87 and 100%) and yields between 0.7 and 1.0 kg m^{-2}. Furthermore, in this system, the co-culture of shrimp/tilapia is also performed to maximize performance. It is still required to demonstrate the technical and economic feasibility of fattening male shrimp in individual containers within seminatural ponds.

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Conflict of interest

The author has no conflict of interest.

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References

[1] Méndez M. Claves de identificación y distribución de los langostinos y camarones (Crustacea: Decapoda) del mar y ríos de la costa del Perú. Boletín Instituto del Mar del Perú. 1981;**5**:1-170

[2] Zacarías S, Yépez V. Camarón de río *Cryphiops caementarius* (Molina, 1782) en la costa centro-sur del Perú, 2007. Informe Instituto del Mar del Perú. 2015;**42**(3):398-415

[3] Produce (Ministerio de Producción). Anuario estadístico pesquero y acuícola 2016. Perú: Ministerio de la Producción; 2017. p. 42

[4] Carrillo AA, Pacora A, Risco RA, Zerpa R. Plan estratégico para el camarón de río [tesis]. Lima: Pontificia Universidad Católica del Perú; 2012

[5] Moscoso V. Catálogo de crustáceos decápodos y estomatópodos del Perú. Boletín Instituto del Mar del Perú. 2012;**27**:1-209

[6] Bahamonde N, Carvacho A, Jara C, López M, Ponce F, Retamal MA, et al. Categorías de conservación de decápodos nativos de aguas continentales de Chile. Boletín del Museo Nacional de Historia Natural del Paraguay. 1998;**47**:91-100

[7] Hobbs HH. Biogeography of subterranean decapods in north and central America and the Caribbean region (Caridea, Astacidea, Brachyura). Hydrobiologia. 1994;**287**:95-104

[8] Villalobos JL, Nates JC, Díaz AC. Revisión de los géneros *Cryphiops* Dana, 1852 y *Bithynops* holthuis, 1973, de la familia Palaemonidae (Crustacea, Decapoda), y descripción de una especie nueva para el estado de Chiapas, México. Anales del Instituto de Biología, Universidad Nacional Autónoma de México. Serie Zoología. 1989;**60**(2):159-184

[9] Gomes MM. Descrição de uma espécie nova do género *Cryphiops* (Decapoda, Natantia, Palaemonidae). Revista Brasileira de Biologia. 1973;**33**(2):169-173

[10] Romano N, Zeng C. Cannibalism of decapod crustaceans and implications for their aquaculture: A review of its prevalence, influencing factors, and mitigating methods. Reviews in Fisheries Science & Aquaculture. 2017;**25**(1):42-69. DOI: 10.1080/23308249.2016.1221379

[11] Mariappan P, Balasundaram C, Schmithz B. Decapod crustacean chelipeds: An overview. Journal of Biosciences. 2000;**25**(3):301-313

[12] Reyes W. Engorde del camarón nativo de los ríos costeros del Perú. Un estudio en sistema de recipientes individuales y con recirculación de agua. Alemania: Publicia; 2016. 89 p

[13] Rodríguez A. Hábitos alimentarios de las jaibas *Callinectes bellicosus* STIMPSON y *C. arcuatus* ORDWAY (Brachiura: Portunidae) en Bahía Magdalena, Baja California Sur México [tesis]. La Paz, Baja California Sur, México: Instituto Politécnico Nacional Centro Interdisciplinario de Ciencias Marinas; 2014

[14] Zúñiga O, Ramos R. Balance energético en juveniles de *Cryphiops caementarius* (Crustacea, Palaemonidae). Biota. 1987;**3**:33-43

[15] Barki A, Harpaz S, Karplus I. Contradictory asymmetries in body and weapon size, and assessment in fighting male prawns, *Macrobrachium rosenbergii*. Aggresive Behavior. 1997;**23**:81-91

[16] Barki A, Karplus I, Goren M. Effects of size and morphotype on dominance hierarchies and resource competition in

the freshwater prawn *Macrobrachium rosenbergii*. Animal Behaviour. 1992;**44**(3):547-555

[17] Santos DB, Pontes CS. Behavioral repertoire of the giant freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879) in laboratory. Journal of Animal Behaviour and Biometeorology. 2016;**4**(4):109-115

[18] Fox LR. Cannibalism in natural population. Annual Review of Ecology and Systematics. 1975;**6**:87-106

[19] Polis GA. The evolution and dynamics of intraspecific predation. Annual Review of Ecology, Evolution, and Systematics. 1981;**12**:225-251

[20] Dennell R. Integument and exoekeleton. In: Waterman TH, editor. The Physiology of Crustacea. Metabolismo and Growth. Vol. 1. Cambridge: Academic Press; 1960. pp. 447-472

[21] Adams JA, Moore PA. Discrimination of conspecific male molt odor signals by male crayfish *Orconectes rusticus*. Journal of Crustacean Biology. 2003;**23**(1):7-14

[22] Tran MV. The scent of cannibalism: The olfactory basis of cannibalism in hermit crabs. Journal of Experimental Marine Biology and Ecology. 2014;**457**:8-14

[23] Ponce JE. Importancia del flujo de agua en los estanques-criaderos de camarón. Actas del Simposio sobre Acuicultura en América Latina, Montevideo, Uruguay, 26 de noviembre a 2 de diciembre de 1974. Documentos de Investigación. FAO, Informes de Pesca. 1977;**1**(159):240-248

[24] Reyes WE. Efecto de dos probióticos bioencapsulados en nauplios de *Artemia franciscana* en el desarrollo larval del camarón de río *Cryphiops caementarius*, en laboratorio [tesis]. Perú: Universidad Nacional de Trujillo; 2008

[25] Shivananda H, Kumarswamy R, Palaksha KJ, Sujatha HR, Shankar R. Effect of different types of shelters on survival and growth of giant freshwater prawn, *Macrobrachium rosenbergii*. Journal of Marine Science and Technology. 2012;**20**(2):153-157

[26] Santos DB, Pontes CS, Campos PMO, Arruda MF. Behavioral profile of *Macrobrachium rosenbergii* in mixed and monosex culture submitted to shelters of different colors. Acta Scientiarum. Biological Sciences. 2015;**37**(3):273-279

[27] Mamun MAA, Hossain MA, Hossain MS, Ali ML. Effects of different types of artificial substrates on nursery production of freshwater prawn, *Macrobrachium rosenbergii* (de Man) in recirculatory system. Journal of the Bangladesh Agricultural University. 2010;**8**(2):333-340

[28] Tuly DM, Islam MS, Hasnahena M, Hasan MR, Hasan MT. Use of artificial substrate in pond culture of freshwater prawn (*Macrobrachium rosenbergii*): A new approach regarding growth performance and economic return. Journal of Fisheries. 2014;**2**(1):53-58

[29] Laranja JLQ, Quinitio ET, Catacutan MR, Coloso RM. Effects of dietary L-tryptophan on the agonistic behavior, growth and survival of juvenile mud crab *Scylla serrata*. Aquaculture. 2010;**310**:84-90

[30] Coyle SD, Alston DE, Sampaio CMS. Nursery systems and management. In: New MB, Valenti WC, Tidwell JH, D'Abramo LR, Kutty MN, editors. Freshwater Prawn. Biology and Farming. United Kingdom: Wiley-Blackwell; 2010. pp. 108-126

[31] Reyes WE, Bacilio S, Villavicencio M, Mendoza R. Efecto de la salinidad en el crecimiento y supervivencia de postlarvas del camarón de río *Cryphiops caementarius* Molina, 1782 (Crustacea, Palaemonidae), en laboratorio.

Comunicación Científica. In: IV Congreso Iberoamericano Virtual de Acuicultura CIVA 2006; Zaragoza, España. 2006. pp. 341-346

[32] Cano F, Carrión S, Reyes W. Efecto de altas densidades de siembra en el crecimiento y supervivencia de postlarvas de *Cryphiops caementarius* (Crustacea: Palaemonidae) en agua salobre. Revista Citecsa. 2014;**5**(8):62-78

[33] Vega-Villasante F, Galavíz-Parada JD, Guzmán-Arroyo M, Flores CA, Espinosa-Chaurand LD. Efecto de diferentes salinidades sobre el crecimiento y supervivencia de juveniles del langostino de río *Macrobrachium tenellum* (Smith, 1871). Zootecnia Tropical. 2011;**29**(4):467-473

[34] Chand BK, Trivedi RK, Dubey SK, Rout SK, Beg MM, Das UK. Effect of salinity on survival and growth of giant freshwater prawn *Macrobrachium rosenbergii* (de Man). Aquaculture Reports. 2015;**2**:26-33. DOI: 10.1016/j.aqrep.2015.05.002

[35] Ponce JE, Eguren MC. Evaluación del crecimiento del camarón de río *Cryphiops caementarius* (Molina) en una poza piloto en Camaná. Véritas. 2005;**9**(1):95-101

[36] Shleser RA. The effects of feeding frequency and space on the growth of the American lobster, *Homarus americanus*. Journal of the World Aquaculture Society. 1974;**5**(1-4):149-155

[37] Van Olst JC, Carlberg JM. The effects on container size and transparency on growth and survival of lobster cultured individually. Proceeding of the Annual Meeting–World Mariculture Society. 1978;**9**(1-4):469-479

[38] Jussila J. Physiological responses of astacid and parastacid crayfishes (Crustacea: Decapoda) to conditions of intensive culture [tesis]. Australia: University of Kuopio; 1977

[39] Manor R, Segev R, Pimenta M, Aflalo ED, Sagi A. Intensification of redclaw crayfish *Cherax quadricarinatus* culture II. Growout in a separate cell system. Aquaculture Engineering. 2002;**26**:263-276

[40] Ford RF, Van Olst JC, Calberg JM, Dorband WR, Johnson RL. Beneficial use thermal effluent in lobster culture. Journal of the World Aquaculture Society. 1975;**6**(1-4):509-519

[41] Kristiansen TS, Drengstig A, Bergheim A, Drengstin T, Svensen R, Kollsgård I, et al. Development of methods for intensive farming of European lobster in recirculated seawater. Results from experiments conducted at Kvitsay lobster hatchery from 2000 to 2004. Fisken og havet. 2004;**6**:1-52

[42] Daniels CL, Wills B, Ruiz-Perez M, Miles E, Wilson RW, Boothroyd D. Development of sea based container culture for rearing European lobster (*Homarus gammarus*) around south west England. Aquaculture. 2015;**448**:186-195

[43] Reyes WE. Crecimiento, reproducción y supervivencia de hembras del camarón de río *Cryphiops caementarius* criados en recipientes individuales. Sciéndo. 2011;**14**(1-2):75-86

[44] Geddes MC, Mills BJ, Walker KF. Growth in the Australian freshwater crayfish *Cherax destructor c*lark, under laboratory conditions. Australian Journal of Marine & Freshwater Research. 1988;**39**:555-568

[45] New MB. Farming freshwater prawn. A manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*). FAO Fisheries Technical Paper. 2002;**428**:1-212

[46] Fuentes AS, Quezada LJ. Efecto de diferentes concentraciones de harina de *Calendula officinalis* "marigold" en la pigmentación de camarones machos adultos de *Cryphiops caementarius* [tesis]. Perú: Universidad Nacional del Santa; 2015

[47] Meyers SP. Papel del carotenoide astaxantina en nutrición de especies acuáticas. In: Civera-Cerecedo R, Pérez-Estrada CJ, Ricque-Marie D, Cruz-Suárez LE, editors. Avances en Nutrición Acuícola IV. Memorias del IV Simposium Internacional de Nutrición Acuícola; 1998; La Paz, B.C.S., México. 2000. pp. 473-491

[48] Wickins JF, Jones E, Beard TW, Edwards DB. Food distribution equipament for individually-housed juvenile lobster (*Homarus* spp.). Aquaculture Engineering. 1987;**6**:277-288

[49] Drengstig A, Bergheim A. Commercial land-based farming of European lobster (*Homarus gammarus* L.) in recirculating aquaculture system (RAS) using a single cage approach. Aquacultural Engineering. 2013;**53**:14-18

[50] Shelley C, Lovatelli A. Mude crab aquaculture. A practical manual. In: FAO Fisheries and Aquaculture Technical Paper. Vol. 567. 2011. pp. 1-78

[51] Pérez RA, Tinoco KL. Cocultivo de machos del camarón de río *Cryphiops caementarius* con ablación del pedúnculo ocular criados en recipientes individuales dentro de tanques con machos de tilapia *Oreochromis niloticus* y su efecto en el crecimiento de las especies [tesis]. Perú: Universidad Nacional del Santa; 2013

[52] Graciano FP, Vásquez JF. Efecto de diferentes niveles de dureza del agua en la muda, crecimiento y supervivencia de adultos del camarón ede río *Cryphiops caementarius* en condiciones de laboratorio [tesis], Perú. Universidad Nacional del Santa; 2015

[53] Cornejo J, Pérez L, Reyes W. Effect of *Saccharomyces cerevisiae* yeast in the diet of male shrimp *Cryphiops caementarius* (Crustacea, Palaemonidae) on total and differential hemocytes count. Revista Bio Ciencias. 2015;**3**(3):173-186

[54] Ramirez M, Cántaro R, Reyes W. Growth and survival of males of *Cryphiops caementarius* (Palaemonidae) with diets supplemented with common salt. Latin American Journal Aquatic Research. 2018;**46**(2):469-474. DOI: 10.3856/vol46-issue2-fulltext-22

[55] Terrones S, Reyes W. Efecto de dietas con ensilado biológico de residuos de molusco en el crecimiento del camarón *Cryphiops caementarius* y tilapia *Oreochromis niloticus* en co-cultivo intensivo. Scientia Agropecuaria, 2018;**9**(2):167-176. DOI: 10.17268/sci.agropecu.2018.02.01

[56] Acosta A, Quiñones D, Reyes W. Efecto de dietas con lecitina de soya en el crecimiento, muda y supervivencia de machos del camarón de río *Cryphiops caementarius* (Crustacea: Palaemonidae). Scientia Agropecuaria. 2018;**9**(1):143-151. DOI: 10.17268/sci.agropecu.2018.01.15

[57] Danaher J, Tidwell J, Coyle S, Dasgupta D. Effects of two densities of caged monosex nile tilapia, *Oreochromis niloticus*, on water quality, phytoplankton populations, and production when polyculture with *Macrobrachium rosenbergii* in temperate pond. Journal of the World Aquaculture Society. 2007;**38**(3):367-382

[58] Mogollón AV. Cocultivo de machos del camarón de río *Cryphiops caementarius* en recipientes individuales dentro de acuario con machos de *Oreochromis niloticus* a diferentes densidades de siembra y sus efectos

en el crecimiento y supervivencia de las especies [tesis]. Perú. Universidad Nacional del Santa; 2013

[59] Coyle SD, Tidwell JH, Yasharian DK, Caporelli A, Skudlarek NA. The effect of biomass density, temperature, and substrate on transport survival of market-size freshwater prawn, *Macrobrachium rosenbergii*. Journal of Applied Aquaculture. 2005;**17**(4):61-71. DOI: 10.1300/J028v17n04_04

[60] Escobar C, Pachamoro MA, Reyes W. Supervivencia y crecimiento de machos adultos del camarón de río *Cryphiops caementarius* Molina 1782 (Crustacea, Palaemonidae) expuestos a salinidades. Ecología Aplicada. 2017;**16**(2):75-82. DOI: 10.21704/rea.v16i2.1010

[61] Ahmed N. Freshwater prawn hatcheries in Bangladesh: Concern of broodstock. Aquaculture Asia Magazine, July-September, 2008. pp. 22-26

[62] Yang G, Frinsko M, Chen X, Wang J, Hu G, Gao Q. Current status of the giant freshwater prawn (*Macrobrachium rosenbergii*) industry in China, with special reference to live transportation. Aquaculture Research. 2012;**43**:1049-1055. DOI: 10.1111/j.1365-2109.2011.03009.x

[63] Fotedar S, Evans L. Health management during handling and live transport of crustaceans: A review. Journal of Invertebrate Pathology. 2011;**106**:143-152. DOI: 10.1016/j.jip.2010.09.011

Chapter 5

Bateman Gradients and Alternative Mating Strategies in a Marine Isopod

Katharine M. Saunders and Stephen M. Shuster

Abstract

The "Bateman gradient" provides a means for estimating the strength of sexual selection. Although widely used for this purpose, this approach has not been applied to examine the covariance between mate numbers and offspring numbers among alternative mating strategies. Differences in this covariance could exist if the average fitnesses of different mating phenotypes were unequal, as has been suggested for "alternative mating tactics." We tested this hypothesis in *Paracerceis sculpta*, a sexually dimorphic marine isopod in which three male morphs coexist. We found no significant differences in sexual competency and no significant differences in Bateman gradients among morphs, that is, the average morph fitnesses were equivalent. However, with data pooled among morphs, we found a significant sex difference in Bateman gradients, as expected for dimorphic species; females gained no additional fitness from mating with multiple males, whereas male fitness increased with increasing mate numbers. In nature, the fitnesses of the three morphs are variable due to differences in the availability of receptive females. Our results suggest that differences in mate availability, not differences in sexual competency, are responsible for observed variance in fitness within, and for the equality of fitnesses among, the three male morphs in this species.

Keywords: measuring sexual selection, male polymorphism, Crustacea, Isopoda

1. Introduction

By definition, females produce few, large ova, whereas males produce many, tiny sperm. This sex difference in initial parental investment is widely viewed as the primary cause of sexual selection and intersexual conflict [1–4]. However, Bateman ([1], p. 363) also argued that, "Variance in number of mates is...the only important cause of the sex difference in the variance in fertility," and therefore that a sex difference in the variance in fertility provides "a measure of the sex difference in intensity of selection." This statement implies that selection within each sex, rather than between the sexes is responsible for sexual selection as well as for the evolution of sexual differences. The magnitude of the sex difference in fitness variance can be specifically quantified, not through proxies for selection intensity,

such as the ratio of sexually mature males to receptive females at any time (the Operational Sex Ratio, OSR [5]) or the ratio of maximum potential reproductive rates for each sex (PRR; [6]), but rather from actual estimates of selection's strength [7–12].

Such measures include the opportunity for selection ($V_W/W^2 = I$; [13]), the ratio of the variance in fitness to its squared average. This parameter, when measured using the mean and variance in mate numbers for each sex and adjusted by the sex ratio, quantifies the sex difference in the opportunity for selection, that is, the opportunity for sexual selection (I_M, [7, 8]; I_{mates} [9]; I_s [14]). Despite an early focus on mate numbers, the opportunity for sexual selection can be measured more precisely using the mean and variance in offspring numbers for each sex [9, 15, 16]. The Bateman gradient, β_{ss} [14, 16–19] provides a more specific estimator of the effect of mating success on fitness, by quantifying the standardized covariance between mate number and offspring number. Jones' Index, $\beta_{ss}\sqrt{I_s}$, combines these parameters and appears to provide a useful correction when the opportunity for sexual selection is expressed in terms of mate numbers rather than in terms of offspring numbers [14, 20].

The Bateman gradient is among the more precise methods for measuring sexual selection because it measures the slope, β_{ss}, of the statistical relationship between mate numbers and offspring numbers for members of each sex [16]. Thus, it estimates the intensity of sexual selection on the trait or traits that influence the sex difference in the variance in offspring numbers, provided that such traits can be identified. Although now widely used to compare sex differences in selection intensity [16–19, 21], the Bateman gradient has not been used to examine the covariance between mate numbers and offspring numbers among polymorphic mating phenotypes, also known as alternative mating strategies [9, 22, 23].

Polymorphisms in mating phenotype are considered by many researchers to provide examples of *fitness satisficing*, a current explanation for why alternative adult morphs persist within populations despite their experience of average fitness that is less than the average fitness of the conventional adult morph. According to this hypothesis, alternative phenotypes appear to "make the best of a bad job" [22–24]. One mechanism by which alternative phenotypes could experience less-than-average fitness is if Bateman gradients among the adult morphs are statistically distinct.

The Gulf of California sphaeromatid isopod, *Paracerceis sculpta*, has three distinct male morphs and breeds within the spongocoels of the sponge, *Leucetta losangelensis*, (**Figure 1**). Alpha males are largest and possess enlarged uropods, used for defending breeding sites. Beta males are smaller than α-males and resemble females in behavior and body form. Gamma males are the smallest and use their small size and agility to "sneak" into spongocoels [25]. Previous results indicate that variance in fitness within each of the three male morphs is large, whereas fitness differences among morphs are minute, a necessary condition for the persistence of genetic polymorphism [26].

While the possible causes of variance in mating success within α-males are relatively well understood [27–32], the causes of within-morph fitness variance for β- and γ-males are less clear. Here, we measured Bateman gradients for α-, β-, and γ-males, and females in *P. sculpta* to determine if there is a significant difference in the covariance between mate numbers and offspring numbers for the four adult phenotypes in this species. Our results reveal the precision of this approach for measuring the difference in sexual selection intensity and suggest an alternative method for investigating fitness differences among morphs in species with sexual polymorphisms.

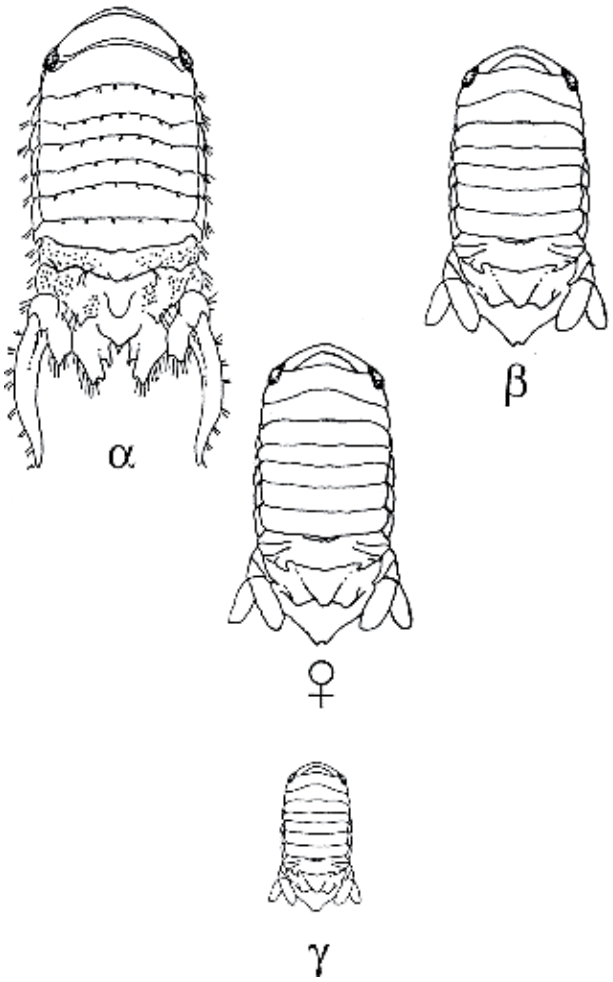

Figure 1.
The α-, β-, and γ-male and female morphs in Paracerceis sculpta (redrawn from Shuster [30]).

2. Materials and methods

2.1 Sexual receptivity, mating, and gestation in *P. sculpta*

Females are attracted to breeding sites in sponges when their ovaries and brood pouches mature [29]. Sexual receptivity in these S_1 females is initiated when they shed the posterior half of their cuticle and expose genital openings at the base of each fifth walking leg [27]. Females in S_2 (half molted) condition remain receptive for 24 h before shedding their anterior cuticles, ovipositing into internal brood pouches and becoming non-receptive (S_3). Females do not feed during gestation (S_4–S_7; [27]). Males complete a mating sequence with receptive females by inserting their appendix masculina and ejaculating into one, and then into the other of their mate's vaginas. Fertilization occurs and zygotes are brooded internally for 3 weeks before being released as fully formed juveniles (mancas; [27–29]).

2.2 Field collections

We collected several hundred isopods from the spongocoels of the intertidal sponge, *Leucetta losangelensis*, in the northern Gulf of California [30]. All individuals

were sexed, scored by reproductive condition, measured to the nearest 0.125 mm, and identified by unique cuticular pigmentation patterns [27, 28]. We retained unmolted, sexually mature (S_1) females (N = 92), as well as α-, β-, and γ-males (N = 41) from samples and placed these individuals into 225-ml plastic cups containing seawater. All other individuals were returned to collection sites within 24 h.

2.3 Matings for males

To examine the relationship between mate number and fertility for the three male morphs, and to compare the fertility of females mated to each of the three male morphs (see below), we allowed α-males (N = 14), β-males (N = 14), and γ-males (N = 13) to mate with 1–5 females in succession ($N_{females}$ = 86). We allowed each male to remain with each female for the duration of her 24-h period of receptivity. We then separated individuals and placed them in separate 225-ml cups containing seawater. Males were then placed with another S_2 female, allowed to mate for 24 h, and the sequence was continued until males either died or mated five times. All S_3 females were maintained in containers until parturition when we counted all mancas and undeveloped zygotes, if present.

To determine whether the fertility of males differed or decreased with increasing mating frequency, as well as to determine whether the fertility of the females mated by α-, β-, and γ-males was statistically distinguishable, we first calculated the residuals for the regression of offspring number on female body size to account for the positive effect female body size has on fertility ($F_{[1,85]}$ = 98.14, P < 0.0001). Then, we analyzed these residuals using a two-way ANOVA to examine the influences of male morph (MORPH), the order of females in the mating queue (ORDER), and their interaction (MORPH*ORDER) on the number of offspring produced by individual females mated by α-, β-, and γ-males. We performed a similar analysis on the number of undeveloped zygotes per female but did not calculate residuals for this analysis because there was no significant relationship between female body length and the number of undeveloped zygotes ($F_{[1,68]}$ = 0.67, P = 0.42).

2.4 Matings for females

To examine the relationship between mate number and fertility for females, we allowed S_2 females to complete one mating sequence each with either 1 (N = 2), 3 (N = 1), or 5 (N = 3) α-males in succession. Pairs of isopods were given a maximum of 20 min to begin mating. To prevent re-mating, we removed males after mating, changed the water in the cup, and allowed each female to recover for 5 min before the next male was introduced. The entire mating sequence for each female never exceeded 2 h. S_3 females were maintained in their containers until parturition, when all mancas were counted. Again, the numbers of undeveloped zygotes, if present, were also counted.

To investigate whether the fertility of females who mated 1–5 times over 2 h, was different from each other as well as from the fertility of the 86 females, we allowed unlimited matings with males over 24 h (see "Matings for males" section), we first calculated the residuals for the regression of offspring number on female body size to account for the positive relationship between female size and fertility ($F_{[1,5]}$ = 15.98, P = 0.02). Next, because of the small sample size of females mated within 2 h (N = 6), we compared the residuals of the fertility of females mated 1, 3 and 5 times using a Kruskal-Wallis test. Because this test was non-significant ($X^2_{[2,6]}$ = 0.86, P = 0.65), we pooled these females for our analysis and compared their fertility as a group, with those of females who were allowed unlimited matings for 24 h. Note that these latter females ($N_{females}$ = 86) were the same females whose fertility was compared when mated with α-, β-, and γ-males above.

Using two-way ANOVA, we then examined the influences of female body length (FBLENG), the time available for mating (DURATION; 1–5 matings in 2 h; unlimited matings in 24 h), and their interaction (FBLENG*DURATION) on the number of offspring produced by females. We performed a similar analysis on the number of undeveloped zygotes per female. As in the previous analysis of undeveloped zygotes, we did not calculate residuals for this analysis because there was no significant relationship between female body length and the number of undeveloped zygotes ($F_{[1,73]} = 1.27$, $P = 0.26$).

2.5 Bateman gradients

We used two-way ANOVA to examine the influences of adult phenotype (ADULTP), mate number (NMATES), and their interaction (ADULTP*NMATES) on the number of offspring produced by α-, β-, and γ-males, and females. We then subdivided our data by sex and used two-way ANOVA to examine the influence of male morph (MORPH), mate number (NMATES), and their interaction (MORPH*NMATES) on the number of offspring produced by α-, β-, and γ-males. Because males were analyzed separately from females, we used a Bonferroni correction to reduce our criterion for significance, $\alpha = 0.05/2 = 0.025$. Lastly, we pooled the data for all males and used two-way ANOVA to examine the influences of sex (SEX), mate numbers (NMATES), and their interaction (SEX*NMATES) on the number of offspring produced by all males and all females. For individual Bateman gradients, we calculated the least squares regression of offspring numbers on mate numbers for each adult morph [16].

3. Results

Our two-way ANOVA of the residuals for offspring number on female body length, to determine whether the fertility of the three male morphs differed or decreased with increasing mating frequency, was non-significant overall ($F_{[5,85]} = 0.25$, $P = 0.94$) with non-significant effects of male morph ($F_{[MORPH]} = 0.42$, $P = 0.66$) and mate order ($F_{[ORDER]} = 2.21$, $P = 0.64$) and a non-significant interaction between these factors ($F_{[MORPH*ORDER]} = 0.15$, $P = 0.86$). This result indicated that the three male morphs did not differ in their sexual competency with multiple matings. This result also confirmed that there were no significant differences in the fertility of females mated with α-, β-, and γ-males, and confirmed that there were no significant differences in the numbers of undeveloped zygotes among females mated by α-, β-, and γ-males ($F_{[5,67]} = 0.18$, $P = 0.97$; $F_{[MORPH]} = 0.31$, $P = 0.73$; $F_{[ORDER]} = 0.01$, $P = 0.95$; $F_{[MORPH*ORDER]} = 0.18$, $P = 0.83$).

Our two-way ANOVA to compare the fertility of females who mated 1–5 times over 2 h vs. the fertility of females allowed unlimited matings over 24 h was significant overall ($F_{[3,81]} = 34.56$, $P < 0.0001$) with a significant effect of body length ($F_{[FBLENG]} = 7.34$, $P = 0.008$), but no significant effect of the time available for mating ($F_{[DURATION]} = 1.03$, $P = 0.31$) and no significant interaction between female body length and the time available for mating ($F_{[FBLENG*DURATION]} = 0.35$, $P = 0.55$). This result indicated that the size-adjusted fertility of females allowed to mate 1–5 times was no different from those of females allowed unlimited access to matings over 24 h. This result was corroborated by our finding that there were no significant differences in the numbers of undeveloped zygotes among females mated 1–5 times compared with females allowed unlimited matings over 24 h. ($F_{[3,73]} = 0.63$, $P = 0.60$; $F_{[FLENG]} = 1.07$, $P = 0.30$; $F_{[DURATION]} = 0.04$, $P = 0.84$; $F_{[FBLENG*DURATION]} = 0.33$, $P = 0.57$).

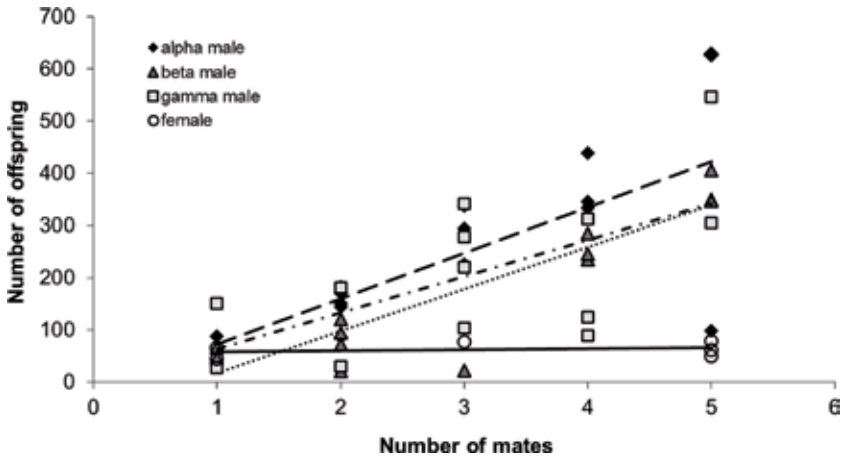

Figure 2.
Bateman gradients estimated for each adult phenotype in P. sculpta: α-males (βss ± SE = 87.60 ± 25.33, N = 14; P = 0.005; black diamonds, dashed line); β-males (βss ± SE = 80.46 ± 11.96, N = 14, P < 0.0001; dark gray triangles, dashed and dotted line); γ-males (βss ± SE = 69.48 ± 26.16, N = 13, P = 0.022; light gray squares, dotted line); females (βss ± SE = 1.78 ± 4.48, N = 6, P = 0.64; open circles, solid line); and pooled males (βss ± SE = 78.92 ± 12.23, N = 41, P < 0.0001; open circles); details of this analysis are described in the text.

Our two-way ANOVA comparing the relationship between mate numbers and offspring numbers for each of the three male morphs and females (**Figure 2**) was significant ($F_{[7, 39]} = 8.71$, $P < 0.001$), with a significant effect of adult phenotype ($F_{[ADULTP]} = 5.13$, $P = 0.004$), a significant effect of mate numbers ($F_{[NMATES]} = 32.60$, $P < 0.0001$), and with a significant interaction between adult phenotype and mate numbers ($F_{[ADULTP*NMATES]} = 3.25$, $P = 0.032$). This result indicated that a phenotype difference in Bateman gradients does exist for *P. sculpta*, but it did not reveal the source of the difference.

That source was revealed by two successive tests. Our two-way ANOVA of males alone, to identify the source of the difference in Bateman gradients among the adult phenotypes, was significant overall ($F_{[5, 35]} = 8.91$, $P < 0.0001$), with a significant effect of mate numbers ($F_{[NMATES]} = 40.66$, $P < 0.0001$). However, we found no significant effect of male morph ($F_{[MORPH]} = 1.59$, $P = 0.22$) and no significant interaction between male morph and mate numbers ($F_{[MORPH*NMATES]} = 0.17$, $P = 0.85$), indicating that Bateman gradients for the three male morphs were indistinguishable. This result justified pooling all males for re-analysis of the relationship between mate numbers and offspring numbers for males and females.

This pooled-male analysis was significant overall ($F_{[3,38]} = 19.09$, $P < 0.001$) with a significant effect of sex ($F_{[SEX]} = 11.81$, $P = 0.001$), a significant effect of mate numbers ($F_{[NMATES]} = 10.14$, $P = 0.003$), and a significant interaction between sex and mate numbers ($F_{[SEX*NMATES]} = 9.26$, $P = 0.004$), a result confirming that a sex difference in Bateman gradients exists for *P. sculpta* (**Figure 2**). In this analysis, the sex difference in the covariance between mate numbers and offspring numbers was over 40-fold larger for males than for females (**Figure 2**).

4. Discussion

Our results showed that although they appear to invest different amounts of energy toward somatic and gametic functions [27, 28], the three male morphs in *P. sculpta* do not differ in their sexual competencies with multiple matings. This result also demonstrated that individual females mated with α-, β-, or γ-males do

not differ in their fertility when allowed to mate with these males *a bene placito* over a 24-h period. Here, we confirmed this finding using the number of live young produced, *as well as* the number of undeveloped zygotes remaining within female brood pouches, thus considering the possibility of the positive, as well as the negative influences that multiple mating may have on female fertility. We also showed that the size-adjusted fertility of females allowed to mate 1–5 times was no different from those of females allowed unlimited access to matings over 24 h. This result justified our comparison of multiple matings by females with multiple matings by males of each of the three male phenotypes in our analysis of Bateman gradients.

Our results further showed that while the three male morphs do not exhibit distinct Bateman gradients, a sex difference in Bateman gradients does exist for *P. sculpta* when adult male and female phenotypes are compared. α-, β-, and γ-males coexist at different population frequencies in nature (α: 0.81; β: 0.15; γ: 0.04; N = 555; [26]) and appear to differ in their mating success in different social circumstances [28, 29]. However, the fact that their Bateman gradients are statistically indistinguishable indicates that under our experimental conditions the fitnesses of the three male morphs were equal. Although the sample size for the females was small relative to females who mated once, as many as five matings had no effect on the number of offspring females in our study produced. Moreover, the fertility of these females, with variable numbers of matings, was no different from the fertility of a larger sample of females (N = 86) with unlimited numbers of matings.

In contrast, within each of the three morphs, male fitness increased linearly with increasing numbers of matings (**Figure 2**). The large difference between the sexes in the number of offspring produced with increased numbers of mates suggests that intersexual conflict (c.f., [1–4, 12]) *could* exist within this species. Indeed in this study, the sex difference in the intensity of selection was over 40 times greater in males than in females (see also [10]). However, the magnitude of this difference also suggests that while intersexual conflict could exist, natural selection on females is considerably weaker than sexual selection on males. An evolutionary response by females to possible sexual exploitation by males, that is, an intersexual arms race of the sort envisioned in intersexual conflict scenarios [1–4, 12], might therefore be undetectable [9]. Despite the possibility of sexual exploitation by males, we found no evidence of that females were negatively affected by multiple matings.

The significant sex difference in Bateman gradients for *P. sculpta* suggests that sexual selection acts much more intensely on males than it does on females in this species. However, this result also indicates that sexual selection does not act differentially among the three male morphs through differences in mate number alone. This result corroborates other results [9, 26] indicating that fitness satisficing does not occur among the male morphs in *P. sculpta* in this context, and that differences in mate availability, not differences in sexual competency, are responsible for observed variance in fitness within, and for the equality of fitnesses among the three male morphs in this species. When β- and γ-males are present with α-males in the spongocoels in which these isopods breed, they tend to be more successful than α-males, particularly when harem sizes are large [9, 26, 28]. These results suggest that β- and γ-males may be more effective in tactics that enhance fertilization success, such as mate guarding or repeated inseminations, than α-males [26].

If this is indeed the case, then as is widely acknowledged, the number of matings individual males acquire need not translate linearly toward that male's overall fitness. More specifically, in nature, multiple Bateman gradients among male morphs may exist that each depend on the number of available mates *as well as* the number of different mating males representing each morph that are present within breeding sites at any given time. Such variation is likely to be widespread among species exhibiting reproductive polymorphisms (reviews in [17, 22, 33–40]). Under

such circumstances, it is unlikely that any given subset of fitness gradients among morphs accurately represents the entire population, particularly if that subset focuses on males who are successful at mating and tends to ignore males who are unsuccessful. Field samples that disproportionately focus on successfully mating males tend to overestimate the fitness of males in the mating class, making it easier to conclude that males expressing alternative mating phenotypes are "making the best of a bad job [9, 40]."

For this reason, we recommend, when male polymorphisms exist, that the fitness for a large number of males of each morph be measured, and their relative fitness outcomes be considered in proportion to the average fitness that all males in the population achieve. This approach is consistent with studies of this and other species [26, 40], in which equal average fitnesses exist among male morphs over multiple generations.

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References

[1] Bateman AJ. Intra-sexual selection in *Drosophila*. Heredity. 1948;**2**:349-368

[2] Williams GC. Adaptation and Natural Selection. Princeton, NJ: Princeton University Press; 1966

[3] Trivers RL. Parental investment and sexual selection. In: Campbell B, editor. Sexual Selection and the Descent of Man. Chicago, IL: Aldine Press; 1972. pp. 136-179

[4] Rubenstein DR, Alcock J. Animal Behavior. 11th ed. New York, Oxford: Sinauer Associates, Oxford University Press; 2019. 548pp

[5] Emlen ST, Oring LW. Ecology, sexual selection, and the evolution of mating systems. Science. 1977;**197**:215-223

[6] Clutton-Brock TH, Vincent ACJ. Sexual selection and the potential reproductive rates of males and females. Nature. 1991;**351**:58-60.7

[7] Wade MJ. Sexual selection and variance in reproductive success. American Naturalist. 1979;**114**:742-764

[8] Wade MJ, Arnold SJ. The intensity of sexual selection in relation to male sexual behaviour, female choice, and sperm precedence. Animal Behaviour. 1980;**28**:446-461

[9] Shuster SM, Wade MJ. Mating Systems and Strategies. Princeton, NJ: Princeton University Press; 2003

[10] Wade MJ, Shuster SM. Don't throw Bateman out with the bathwater! Integrative and Comparative Biology. 2005;**45**:261-268

[11] Krakauer AH, Webster MS, DuVal EH, Jones AG, Shuster SM. The opportunity for sexual selection: Not mismeasured, just misunderstood. Journal of Evolutionary Biology. 2011;**24**:2064-2071

[12] Kokko H, Klug H, Jennions MD. Unifying cornerstones of sexual selection: Operational sex ratio, Bateman gradient and the scope for competitive investment. Ecology Letters. 2012;**15**:1340-1351

[13] Crow JF. Some possibilities for measuring selection intensities in man. Human Biology. 1958;**30**:1-13

[14] Jones AG. On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. Evolution. 2009;**63**:1673-1684

[15] Moorad JA, Wade MJ. Selection gradients, the opportunity for selection and the coefficient of determination. The American Naturalist. 2013;**181**:291-300

[16] Arnold SJ, Duvall D. Animal mating systems: A synthesis based on selection theory. American Naturalist. 1994;**143**:317-348

[17] Jones AG, Rosenqvist G, Anders B, Arnold SJ, Avise JC. The Bateman gradient and the cause of sexual selection in a sex-role-reversed pipefish. Proceedings of the Royal Society of London B. 2000;**267**:677-680

[18] Jones AG, Arguello JR, Arnold SJ. Molecular parentage analysis in experimental newt populations: The response of mating system measures to variation in the operational sex ratio. American Naturalist. 2004;**164**:444-456

[19] Jones AG, Rosenqvist G, Berglund A, Avise JC. The measurement of sexual selection using Bateman's Principles: An experimental test in the sex-role reversed pipefish *Syngnathus typhle*. Integrative and Comparative Biology. 2005;**45**:874-884

[20] Henshaw JM, Kahn AT, Fritzche K. A rigorous comparison of sexual selection indexes via simulations of diverse mating systems. Proceedings of the National Academy of Sciences. 2016;**113**:E300-E308

[21] Bjork A, Pitnick S. Intensity of sexual selection along the anisogamy-isogamy continuum. Nature. 2006;**441**:742-748

[22] Gross MR. Alternative reproductive strategies and tactics: Diversity within sexes. Trends in Ecology & Evolution. 1996;**11**:92-97

[23] Tomkins JL, Hazel W. The status of the conditional evolutionary stable strategy. Trends in Ecology & Evolution. 2007;**22**:522-528

[24] Dawkins R. Good strategy or evolutionary stable strategy? In: Barlow GW, Silverberg J, editors. Sociobiology: Beyond Nature/ Nurture? Boulder, CO: Westview; 1980. pp. 331-367

[25] Shuster SM. Alternative reproductive behaviors: three discrete male morphs in *Paracerceis sculpta*, an intertidal isopod from the northern Gulf of California. Journal of Crustacean Biology. 1987;**7**(2):318-327

[26] Shuster SM, Wade MJ. Equal mating success among male reproductive strategies in a marine isopod. Nature. 1991;**350**:608-610

[27] Shuster SM. Female sexual receptivity associated with molting and differences in copulatory behavior among the three male morphs in *Paracerceis sculpta* (Crustacea: Isopoda). Biological Bulletin. 1989;**177**:331-337

[28] Shuster SM. Male alternative reproductive behaviors in a marine isopod crustacean (*Paracerceis sculpta*): The use of genetic markers to measure differences in fertilization success among α-, β- and γ-males. Evolution. 1989;**34**:168-169

[29] Shuster SM. Courtship and female mate selection in a semelparous isopod crustacean (*Paracerceis sculpta*). Animal Behaviour. 1990;**40**:390-399

[30] Shuster SM. The reproductive behaviour of α-, β- and γ-males in *Paracerceis sculpta*, a marine isopod crustacean. Animal Behaviour. 1992;**121**:231-258

[31] Shuster SM, Arnold EM. The effect of females on male-male competition in atypical breeding habitats in the marine isopod, *Paracerceis sculpta*: A reaction norm approach to behavioral plasticity. Journal of Crustacean Biology. 2007;**27**(3):417-424

[32] Shuster SM. The expression of crustacean mating strategies. In: Brockmann HJ, Olivieri R, Taborsky M, editors. Alternative Mating Tactics. Cambridge, UK: Cambridge University Press; 2008

[33] Gross MR, Charnov EL. Alternative male life histories in bluegill sunfish. Proceedings of the National Academy of Science, USA. 1980;**77**:6937-6948

[34] Thornhill R. Panorpa (Mecoptera: Panorpidae) scorpionflies: Systems for understanding resource-defense polygyny and alternative male reproductive efforts. Annual Review of Ecology and Systematics. 1981;**12**:355-386

[35] Lank DB, Smith CM, Hanotte O, Burke T, Cooke F. Genetic polymorphism for alternative mating behaviour in lekking male ruff. Nature. 1995;**378**:59-62

[36] Sinervo B. Selection in local neighborhoods, graininess of social environments, and the ecology of

alternative strategies. In: Dugatkin LA, editor. Model Systems in Behavioral Ecology. Princeton, NJ: Princeton University Press; 2001. pp. 191-226

[37] Tomkins JL, Brown GS. Population density drives the local evolution of a threshold dimorphism. Nature. 2004;**431**:1099-1103

[38] Isvaran K. Variation in male mating behaviour within ungulate populations: Patterns and processes. Current Science. 2005;**89**:1192-1199

[39] Beveridge M, Simmons LW, Alcock J. Genetic breeding system and investment patterns within the nests of Dawson's burrowing bee (*Amegilla dawsoni*) (Hymenoptera: Anthophorini). Molecular Ecology. 2006;**15**:3459-3467

[40] Shuster SM, Willen RM, Keane B, Solomon NG. Alternative mating tactics in socially monogamous prairie voles (*Microtus ochrogaster*). Frontiers in Ecology and Evolution. 2019;**7**(7):1-19

Chapter 6

The Habitat Types of Freshwater Prawns (Palaemonidae: *Macrobrachium*) with Abbreviated Larval Development in Mesoamerica (Mexico, Guatemala and Belize)

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Abstract

The freshwater prawns of genus *Macrobrachium* with abbreviated larval development have been reported from a diversity of freshwater habitats (caves, springs and primary streams from so-long basins). Here we analysed 360 sites around the Mesoamerican region (Mexico, Guatemala and Belize). At each site, we measured temperature, salinity oxygen dissolved, pH, altitude and water flow velocity values. We documented the riparian vegetation and occurrence and abundance of *Macrobrachium* populations. All these values were analysed by multi-dimensional scaling and principal components analysis in order to identify key features of the environmental data that determine the habitat types and habitat diversity. The results show that there are *Macrobrachium* populations in 70 sites inhabiting two main habitats: Lotic and Lentic; and each one have fours subhabitat types. All are defined by altitude range and water velocity that involve the temperature and oxygen variables. In some specific areas, the karstic values on salinity and pH defined some groups. Within the lentic habitats, we identified the following subhabitats: (1) temperate streams, (2) neutral streams, (3) high dissolved oxygen, (4) multifactorial; and for lotic habitats, we identified: (5) water high carbonate, (6) moderate dissolved oxygen, (7) low dissolved oxygen, and (8) high altitude streams. All these subhabitats are located on the drainage basin to the Atlantic Sea, including places from 50 to 850 meters above sea levels and have specifically ranges from temperature, water velocity, pH and salinity for some cases. Also, the geological analysis from the basins where the *Macrobrachium* inhabit is located showed that the geological faults align with these habitat subdivisions. In this chapter, we discuss the environmental heterogeneity, morphological plasticity and their relationship to physiographic regions across the species ranges.

Keywords: *Macrobrachium*, abbreviated larval development, Mesoamerica, habitat

1. Introduction

The freshwater prawns of the genus *Macrobrachium* are characterized by living in the circumtropical region around the world, since these decapods have been reported from the five continents [1]. There are two main groups of freshwater prawns: (a) those that migrate at some point in their life looking for brackish water (amphidromy) and (b) those that are completely hololimnetic and that live restricted to the river springs or caves or grottoes [2–6]. In this chapter, we will cover the different habitat types from the freshwater shrimp that do not reach large sizes (abbreviated larval development) but are of great importance for local or indigenous cultures of the regions where they are found [7]. These organisms represent a great diversity of habitats (caves, primary rivers, creeks, and springs) as well as species (**Figure 1**). In 1999 *Macrobrachium tuxtlaense*, the first epigeous species, was reported from Mexico, and since then seven species have been recorded from Mexico (*M. vicconi*, *M. totonacum*, *M. sbordonii*, *M. cosoloapaensis*, *M. jacatepecensis*, *M. mazatecum*, *M. oaxensis*). In 2015, the first one was reported from Guatemala (*M. cemai*), which led to the study that is now being presented, including Belize.

Figure 1.
Diversity of freshwater prawns in Mesoamerica.

In general, these organisms had been reported between 100 and 500 meters from rivers, streams, and caves in the tropics and only on the Atlantic slope [1, 8–11]. A cave species with this type of development has been known (*Macrobrachium villalobosi* [12]) since 1973, and the last described species of this type of habitat was in 2008 (*M. sbordonii* [13]). Now several environmental data from caves in Mexico, Guatemala, and Belize are available for analysis. Our study shows with more specific detail how the habitats from these freshwater prawns are characterized by diverse environmental conditions that drive high plasticity in their morphological features.

2. Material and methods

The study area focused on the Southeast of Mexico with Veracruz, Oaxaca, Tabasco, and Chiapas States, as well as the Alta Verapaz, Peten, and Lake Izabal regions in Guatemala and the region mountain range that drains into the Atlantic Ocean in Belize (**Figure 2**). This study was carried out from 2006 to 2018. Throughout this period, sites were visited one time where we searched for freshwater prawns and recorded environmental data. We sampled from all of these regions (rivers, streams, springs, and caves) that had the following characteristics: (1) between 800 and 100 meters above sea level (masl), (2) a tropical origin, and (3) predominantly freshwater. We sampled 360 sites measuring the following ecological features with an Oximeter Oakton: dissolved oxygen (±0.01 mg/l), pH (±001 pH), salinity (±0.01%), and temperature of the water (±0.01°C). The altitude and the GPS values were recorded with a Garmin GPS, and the speed of the surface current was recorded taking the speed at which a floating object takes to travel a known distance. After the environmental data were recorded, the prawns were collected

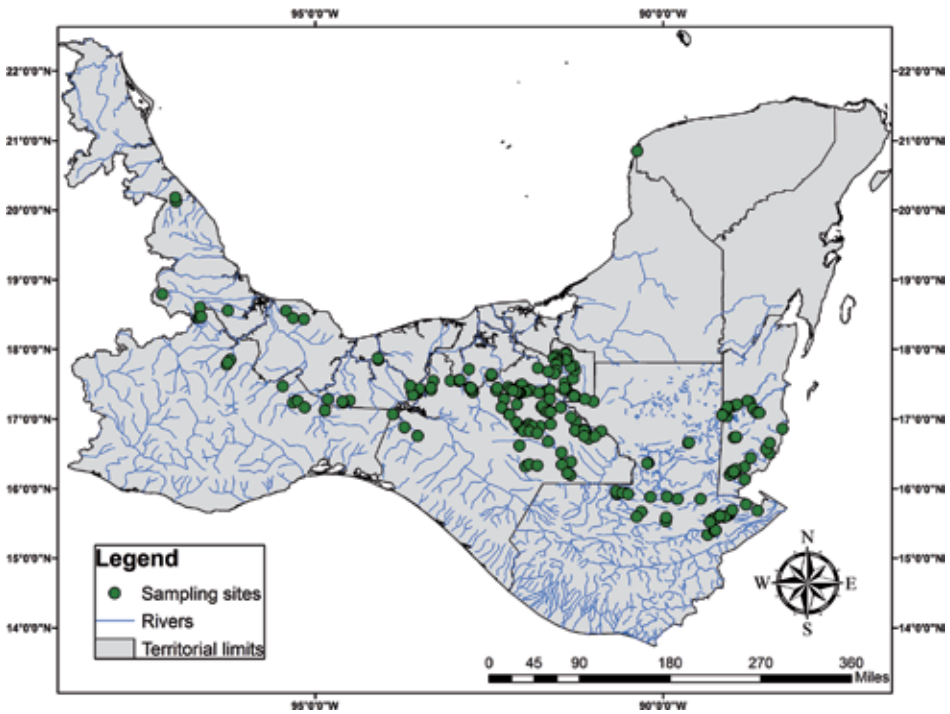

Figure 2.
Sampling sites around Mesoamerica.

with a target number of at less 30 animals, including larger animals with few eggs as indicators of abbreviated larval development. Animals were collected using a net or manually according to the conditions of the site; for example, in the ponds with lentic features, the manually sampled was used, while in the lotic features, the net was the better method. The animals were identified according to genus using the taxonomic keys described previously [11]. The first step was a basin analysis using the geographic information system (GIS) procedure to identify the stream morphometric traits in the different basins where these animals occur (tectonic faults and hypsometric slopes) [14].

Likewise, we analyzed habitat types using the software Primer 6 [15]. All these values were standardized and analyzed by nonparametric multidimensional scaling (MDS) using the Kruskal-Wallis test and principal component analysis (PCA) procedures [16], in order to identify which environmental characteristics determine the sub-habitat types and how many types are possible to identify.

3. Results

3.1 Sites with freshwater prawns

Freshwater prawns with abbreviated larval development were found in 70 sites from 360, analyzed, and distributed mainly between 100 and 860 meters above sea level. In all cases the freshwater environments originated in tropical areas. The species found were just mentioned above together with three more cave species: *Macrobrachium villalobosi*, *M. acherontium*, and *M. catonium*. However, from the 70 populations, we believe there are at less 15 new species that are currently in the process description. All these populations show a pattern of distribution along the mountain range of the Sierra Madre Oriental and the Sierra Madre de Chiapas reaching the region of Alta Verapaz and Peten until ending at Lake Izabal through the Guatemalan territory and the Belizean mountain mass in the rivers that drain to the Caribbean and therefore to the Atlantic Ocean (**Figure 3**). In general, the sites can be characterized as freshwater environments (cave or epigeal) and springs or primary streams. Their distribution is restricted to the presence of large volumes of water and higher current flow, as well as the presence of migratory species of prawns (amphidroms) that are generally larger with a lot of eggs. In a basin analysis with the sites where *Macrobrachium* shrimp were found, our results show that most populations live in areas with tectonic faults, where one finds the majority of the springs and caves and only few populations live in areas where the deposition and erosion processes are dominant.

3.2 Cluster analysis

In the first grouping analysis with the most specific data, we found two higher-order habitat divisions, namely, lotic and lentic sites (**Figure 4**). But within each higher-order environment, there were four subtypes of habitat with the subterranean habitat not clearly partitioned between lotic and lentic but was more frequent in lentic environments: (1) temperate streams, (2) neutral streams, (3) high dissolved oxygen, and (4) multifactorial habitats. And for lotic habitats, we identified the following sub-habitats: (5) water high carbonate, (6) moderate dissolved oxygen, (7) low dissolved oxygen, and (8) high-altitude streams (see **Table 1**).

Some sub-habitats overlapped with each other, but others are well defined and are separated from others (**Figure 5**). These overlapping habitats occurred in 3, 4,

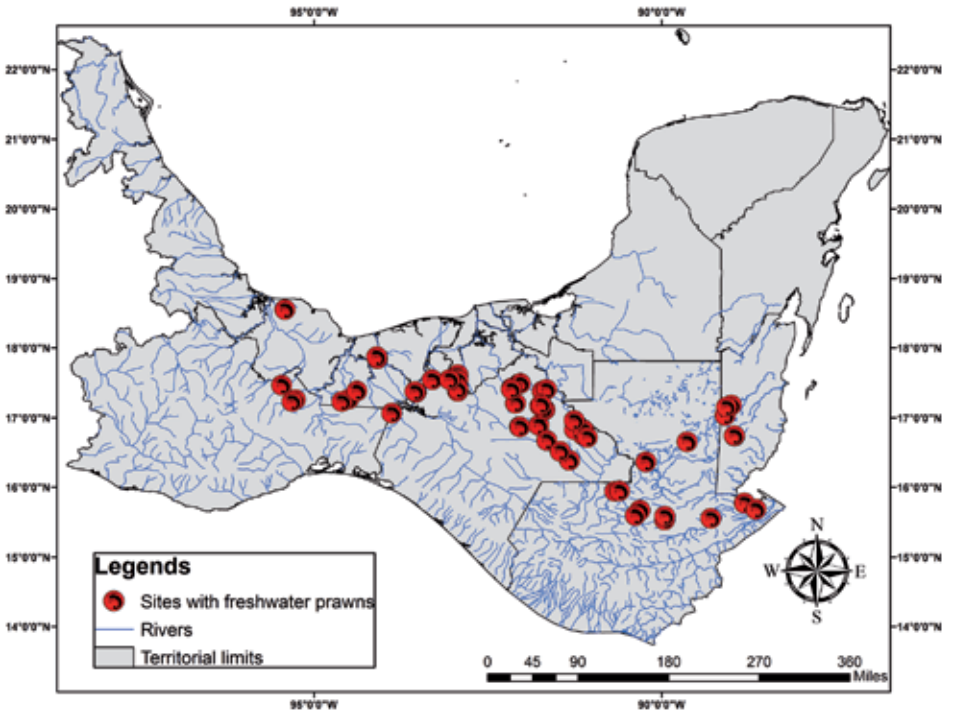

Figure 3.
Sites with freshwater prawns along Mesoamerica.

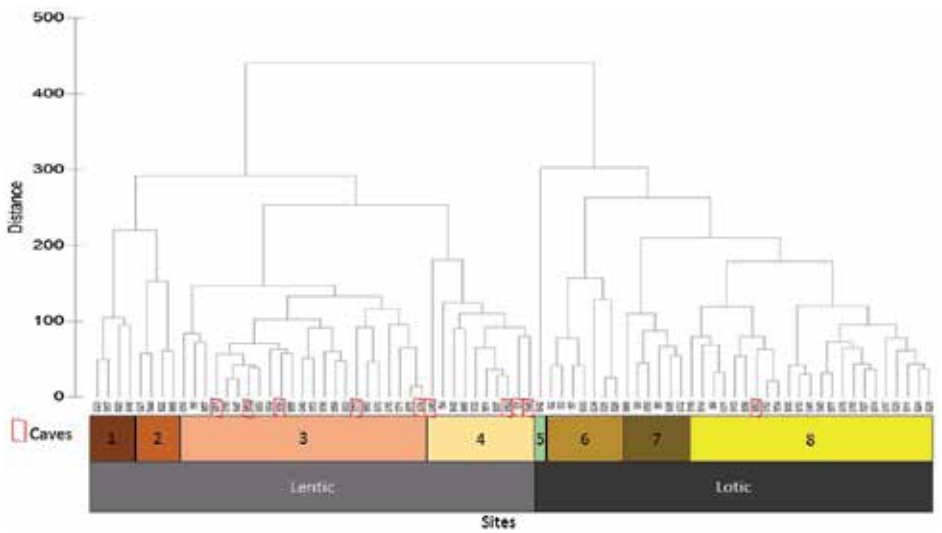

Figure 4.
Cluster analysis using the nearest neighbor method and Euclidean distance (normalized data). Within lentic habitats, the following sub-habitats were identified: (1) temperate streams, (2) neutral streams, (3) high oxygen dissolved, and (4) multifactorial habitats. And for lotic habitats, the following were identified: (5) water high carbonate, (6) moderate dissolved oxygen, (7) low dissolved oxygen, and (8) high-altitude streams.

6, 7, and 8 groups. Likewise, each of these sub-habitats can be separated first by altitude as a predominant variable and then by dissolved oxygen and temperature as well as the current water flow that involves the first classification as lentic or lotic. In general, it is understandable the interrelation of the sub-habitats shares some

Sub-habitat	Characteristics
(1) Temperate streams	They are streams with high temperatures of 25.3–32.4°C; they are at an altitude of <353 masl, have a pH of 6.38–7, are mostly epigeous-type environments, and have an oxygen saturation <34.7% with the exception of a site that presented super saturation
(2) Neutral streams	They are streams with a pH close to neutrality; they exist at altitudes <280 masl, temperatures from 25.2–28.3°C, epigeous-type environments; and they have an oxygen saturation of 11.7–87%
(3) High-oxygen streams	They are streams that mostly have oxygen saturation >60% reaching over saturation in several places. They are found at altitudes of 9–599 masl, temperatures of 18.7–27.7°C, in most epigeal environments although we can find hypogeal representatives
(4) Multifactorial	Streams in which we can find both epigeal and hypogeal sites; lentic and predominantly lotic environments; at altitudes from 34 to 330 masl, temperatures of 22.5–28.2°C, oxygen saturation of 10.3–90.6%, and have a pH of 5–8.3
(5) Water high carbonate	Site with freshwater stream with a height of 371 masl; originating from a karstic cave with a dissolution of the rock higher than other caves, which allows the conductivity to increase and the site to be separated from the rest by values of salinities of 0.9%, with an alkaline pH, a temperature of 22.7°C in a lotic and epigeous environment, and a current speed of 6.6 cm/sec and oxygen saturation of 48.6%
(6) Moderate-oxygen streams	Streams with an oxygen saturation of 38.2–60.5%, having an average altitude of 301 masl and a temperature of 21–24.6°C. They are epigeous environments with an average current speed of 4.8 cm/sec
(7) Few oxygen dissolved	Streams with oxygen saturation of 29.4–46.8%; they have an average altitude of 25.6 masl and a temperature of 20.6–27.1°C and are epigeous environments with an average pH of 6.45
(8) High-altitude streams	Streams with an average altitude of 425.5 masl with a maximum of 844 masl. Oxygen saturation of 31.6–96.9% and a pH of 6.03 at 8.21

Table 1.
Sub-habitat types of Macrobrachium *populations in the Mesoamerican region.*

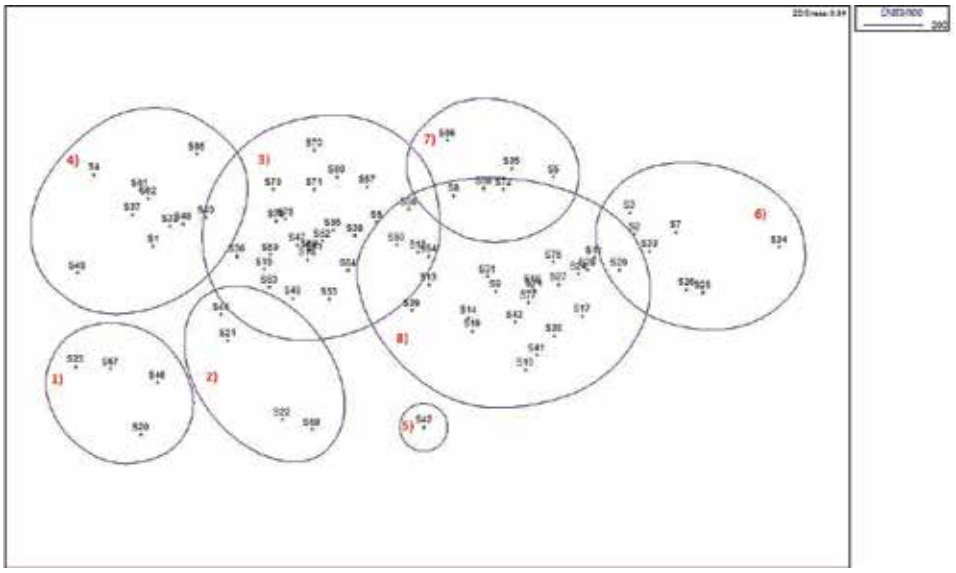

Figure 5.
Nonparametric multidimensional scaling (MDS) analysis using the Euclidean distance at 0.01% stress with standardized data, showing the sub-habitat types of Macrobrachium *populations in Mesoamerica. Within lentic habitats the following sub-habitats were identified: (1) temperate streams, (2) neutral streams, (3) high oxygen dissolved, and (4) multifactorial habitats. And for lotic habitats, the following were identified: (5) water high carbonate, (6) moderate dissolved oxygen, (7) low dissolved oxygen, and (8) high-altitude streams.*

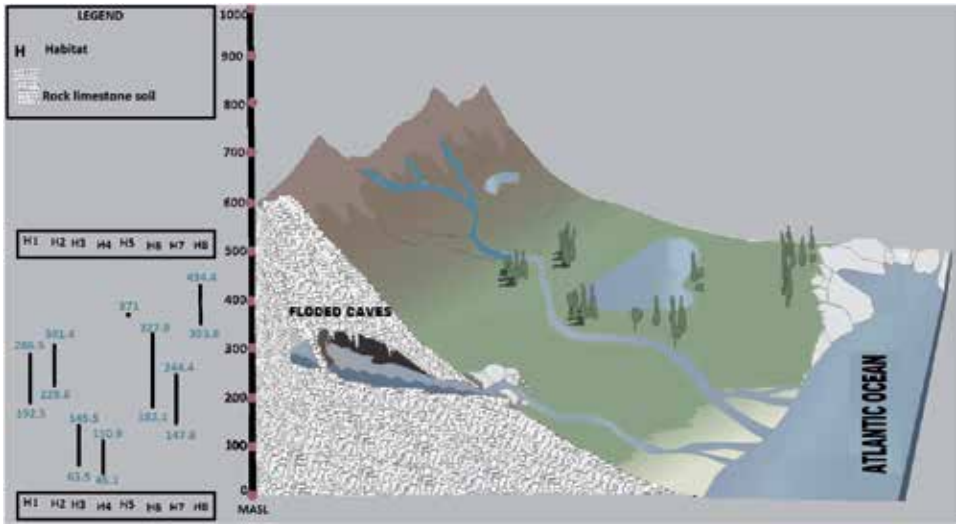

Figure 6.
Diagrammatic scheme of sub-habitat types of Macrobrachium *populations across the coastal slope of Mesoamerica.*

sites with similar characteristics because they are all in the same altitude range as well as the characteristics of tropical springs and rivers.

The schematic representation in the coastal slope of the sub-habitats and their presence is defined by the altitude above sea level, as well as by the water flow and the higher presence of carbonate salts for a specific point such as the case of sub-habitat 5 (**Figure 6**). It is important to mention that most of the places where these organisms live are located in the tectonic faults of the different regions and few populations are found in the river areas where sedimentation and erosion are the main processes of formation of these basins. This makes sense when listing the six general characteristics of the habitats of these organisms: (1) tropical rivers, (2) altitude ranges from 50 to 850 meters above sea level, (3) mainly tectonic faults (springs, primary rivers, and caves), (4) preferably rich in oxygen, (5) with riparian arboreal vegetation, and (6) on the Atlantic slope with karstic origin.

4. Discussion

According to their distributional range, the freshwater prawns with abbreviated larval development inhabit areas from 50 to 600 masl; however, there are some extreme values in the lower range down to 9 masl and up to 840 masl. All species are located along the mountain systems of the Atlantic slope in Mexico, Guatemala, and Belize in contrast with those species of amphidromous prawns that need to migrate along the rivers to reach the brackish waters to complete their development such as *Macrobrachium carcinus*, *M. heterochirus*, *M. olfersii*, *M. americanum*, and *M. acanthurus* [1, 6, 22]. The genus *Macrobrachium* has their origins estimated to have occurred in the Cretaceous [17] and just is when these mountain systems arose, with the volcanic belt serving as a geographical barrier for this group. The *Macrobrachium* species colonized these freshwater habitats together with *Creaseria morleyi* before the emergence of the Yucatan Peninsula [18]. Because the diversification among the freshwater prawns with abbreviated larval development occurred from an ancestor previously adapted [19] to the freshwater habitats, the isolation of suitable freshwater conditions (cooler temperatures, etc.) worked as driving

force in the diversification of these species. With the distributional pattern now established, resolving relationships among these species and populations will aid in testing hypotheses concerning the patterns and timing of diversification events across this group.

Here we distinguish eight sub-habitat types where the freshwater prawns with abbreviated larval development occur. Lentic habitats had the following characteristics: (1) temperate streams, (2) neutral streams, (3) high dissolved oxygen, and (4) multifactorial habitats. And the lotic habitats had the following characteristics: (5) water high carbonate, (6) moderate dissolved oxygen, and (7) low dissolved oxygen. Finally, the last sub-habitat was represented by (8) high-altitude streams independent of epigeal or subterranean populations. This classification is the most detailed analysis across a large region, allowing us to propose six general characteristics of the habitats of these organisms: (1) tropical rivers, (2) altitude ranges from 50 to 850 meters above sea level, (3) mainly tectonic faults (springs, primary rivers, and caves), (4) preferably rich in oxygen, (5) with riparian arboreal vegetation, and (6) on the Atlantic slope with karstic origin. As a result, few sites were recorded where these species coexisted with another *Macrobrachium* species (those with complete larval development species; specifically with *M. carcinus* and *M. olfersii*). In general, all sites are clean without records of pollutants both solids and dissolved. These freshwater shrimp with abbreviated larval development are preferentially distributed in areas where these amphidromous shrimps do not live because the amphidroms are predators of the abbreviated development shrimps. As a result, the abbreviated development shrimps have a microdistribution in each hydrological basin that is limited to the primary rivers and springs where species of amphidroms cannot migrate as it has been invariably reported in different species [10, 20–23]. Our analysis shows that even on a small scale, multiple environmental factors define not only the sub-habitats within lotic or lentic areas but even the differences within and among springs, primary streams, or caves. Consequently, we infer that *Macrobrachium* species are not only plasticity in the morphological traits as has been in another papers, but this plasticity extends to their habitat requirements with oxygen dissolved potentially being more important than even light or temperature. However, if the freshwater prawns of the genus *Macrobrachium* can be so specific to live in certain sub-habitats, this also makes them vulnerable to a such changing environment and so exposed to pollution due to the increase of wastewater or the disappearance of these tropical rivers, given the demand for drinking water by humans. Thus, monitoring of these habitats and especially dissolved oxygen levels is encouraged for the protection of these species.

Acknowledgements

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References

[1] Anger K. Neotropical *Macrobrachium* (Caridea: Palaemonidae): On the biology, origin, and radiation of freshwater-invading shrimp. Journal of Crustacean Biology. 2013;**33**(2):151-183

[2] Jalijal DR, Sankolli KN, Shenoy S. Evolution of larval developmental patterns and the process of freshwaterization in the prawn genus *Macrobrachium* bate, 1868 (Decapoda, Palaemonidae). Crustaceana. 1993;**65**(3):365-376

[3] Signoret GPB, Ortega ALM, Brailovsky DS. Partially abbreviated larval development in an undescribed freshwater palaemonid prawn of the genus *Macrobrachium* from Chiapas, Mexico. Crustaceana. 2000;**73**(3):273-282

[4] Alvarez F, Villalobos JL, Robles F. Abbreviated larval development of *Macrobrachium tuxtlaense* Villalobos and Alvarez, 1999, reared in the laboratory. Crustaceana. 2002;**75**:717-730

[5] Mejía-Ortíz LM, Hartnoll RG, López-Mejía M. The abbreviated larval development of *Macrobrachium totonacum* Mejia, Alvarez and Hartnoll, 2003 (Decapoda, Palaemonidae), reared in the laboratory. Crustaceana. 2010;**83**(1):1-16

[6] Bauer RT. Amphidromy in shrimp: A life cycle between rivers and the sea. Latin American Journal of Aquatic Research. 2013;**41**(4):633-650

[7] Mejia-Ortiz LM, López-Mejía M, Chale Chim A, Perera-Pech Y, Crandall KA, Frausto-Martínez O, et al. Freshwater prawns (Palaemonidae: *Macrobrachium*) with abbreviated larval development in rivers of Mexico: Uses, management and conservation opportunities. In: Bucur D, editor. Basin River Management. Croatia: Intech; 2016

[8] Villalobos JL, Alvarez F. A new species of *Macrobrachium* (Crustacea: Decapoda: Palaemonidae), with abbreviated development, from Veracruz, Mexico. Proceedings of the Biological Society of Washington. 1999;**112**(4):746-753

[9] Roman R, Ortega AL, Mejía LM. *Macrobrachium vicconi*, a new species of fresh-water shrimp from a rain forest in Southeast Mexico, and comparison with congeners (Decapoda, Palaemonidae). Journal of Crustacean Biology. 2000;**20**(1):186-194

[10] Mejía LM, Álvarez F, Hartnoll RG. A new species of freshwater prawn, *Macrobrachium totonacum* (Decapoda: Palaemonidae), with abbreviated development from Mexico. Crustaceana. 2003;**76**:77-86

[11] Mejía-Ortíz LM, López-Mejía M. Freshwater prawns of the genus *Macrobrachium* (Decapoda, Palaemonidae) with abbreviated development from the Papaloapan River basin, Veracruz, Mexico: Distribution and new species. Crustaceana. 2011;**84**(8):949-973

[12] Hobbs HH Jr. Two new troglobitic shrimps (Decapoda: Alpheidae and Palaemonidae) from Oaxaca, México. Bulletin of the Association for Mexican Cave Studies. 1973;**5**:73-80

[13] Mejía-Ortíz LM, Baldari F, López-Mejía M. *Macrobrachium sbordonii* (Decapoda: Palaemonidae), a new stygobitic species of freshwater prawn from Chiapas Mexico. Zootaxa. 2008;**1814**:49-57

[14] Racca JM. Análisis hipsométrico, frecuencia altimétrica y pendientes medias a partir de modelos digitales del terreno. Boletín del Instituto de Fisiografía y Geología. 2007;**77**(1-2):31-38

[15] López-Mejía M. Diversificación de Procambarus (Villalobosus) Hobbs, 1972 (Crustacea: Decapoda: Cambaridae) (Tesis de Doctorado). UNAM: Instituto de Ciencias del Mar y Limnología; 2006. p. 227

[16] Oksanen J. Multivariate Analysis of Ecological Communities in R: Vegan Tutorial. 2015. Available from: http://cc.oulu.fi/~jarioksa/opetus/metodi/vegantutor

[17] Porter ML, Pérez-Losada M, Crandall KA. Model-based multi-locus estimation of decapod phylogeny and divergence times. Molecular Phylogenetics and Evolution. 2005;**37**:355-369

[18] Botello A, Álvarez F. Phylogenetic relationships among freshwater genera of palaemonid shrimps (Crustacea: Decapoda) from Mexico: Evidence of multiple invasions? Latin American Journal of Aquatic Research. 2013;**41**(4):773-780

[19] Murphy NP, Austin CM. Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae): Biogeography, taxonomy and the convergent evolution of abbreviated larval development. Zoologica Scripta. 2005;**34**(2):187-197

[20] Bauer RT, Delahoussaye J. Life history migrations of the amphidromous river shrimp *Macrobrachium ohione* from a continental large river system. Journal of Crustacean Biology. 2008;**28**(4):622-632

[21] Holthuis LB. Cave shrimp (Crustacea, Decapoda, Natantia) from Mexico. Accademia Nazionale dei Lincei. 1977;**171**:173-195

[22] Mejía-Ortíz LM, Alvarez F, Román R, Viccon-Pale JA. Fecundity and distribution of freshwater prawns of the genus *Macrobrachium* in the Huitzilapan river, Veracruz, Mexico. Crustaceana. 2001;**74**(1):69-77

[23] Mejía-Ortíz LM, Álvarez F. Seasonal effects on the distribution of three species of *Macrobrachium* along an altitudinal gradient. Crustaceana. 2010;**83**(4):385-397

Section 2

Fisheries

Chapter 7

Estimation of the Maximum Sustainable Yield and the Optimal Fishing Effort of the Blue Crab (*Callinectes sapidus*, Rathbun 1896) of Laguna Madre, Tamaulipas, Mexico

Jorge Homero Rodriguez Castro, Sandra Edith Olmeda de la Fuente, Wanda Ortiz Baez, Alfonso Correa Sandoval and Jose Alberto Ramirez de León

Abstract

The fishery of the blue crab (*Callinectes sapidus*) in Laguna Madre (LM), Tamaulipas, Mexico, with an average annual catch of 3307 tons, is of great importance economically and socially. The objective of this research was to estimate the carrying capacity (K), the catchability coefficient (q), the maximum sustainable yield (MSY) (tons), and the optimal fishing effort (f_{MSY}) (traps). For this, a time series from 1998 to 2012 was used for the catch and number. The Fox (1970) and Schaefer (1954) models included in A Surplus-Production Model Incorporating Covariates (ASPIC) software were employed for this study. A set of statistical variability estimators and the Akaike's, Bayesian, and Hannan-Quinn information criteria were used for the selection of models. The results obtained by the fox model were K = 54,000, q = 0.00008798, MSY = 2567 and f_{MSY} = 146,900 traps, whereas for the Schaefer model, the results were K = 28,370, q = 0.00002425, MSY = 2008, and f_{MSY} = 58,390. The model with the best adjustment was that of Schaefer. It is concluded that the fishing resource has been overexploited during the period 2003–2011, with an average annual surplus of 670 tons and 25,000 traps. It is recommended to consider the MSY and f_{MSY} values of the Schaefer model for the National Fishing Charter (NFC).

Keywords: *Callinectes sapidus*, blue crab, Laguna Madre, Mexico, maximum sustained yield, ASPIC

1. Introduction

In 2017, a total of 48,602 tons of blue crab was captured in Mexico, 4033 of which came from the State of Tamaulipas. Such state capture allows the State to

occupy the fifth place at a national scale, thus taking the fifth place among the nine main fisheries of the State, according to the definition of the Yearbook of Fishery and Aquaculture Statistics 2017 [1]. An estimate of 3307 tons from the capture the State of Tamaulipas comes from Laguna Madre (LM). This goes in accordance with the proportion of 0.82 that corresponds to LM from the total capture of the blue crab in Tamaulipas, according to Rodríguez-Castro et al. [2]. In economic terms the value (in Mexican pesos and its equivalent in US dollars) of the capture of the blue crab, corresponding to the year 2017, was 51.26 million pesos (2.44 million US dollars) for the Laguna Madre; 62.51 million pesos (2.98 million US dollars) for the State of Tamaulipas; and 753.33 million pesos (35.87 million US dollars) for the country.

The SEMARNAT [3] indicates that the blue crab fishery of LM forms part of the group of fisheries that concurs in the natural protected area, named as *Área de Protección de Flora y Fauna Laguna Madre y Delta del Río Bravo*, and thus is an important economic source in the zone. Furthermore, the SEMARNAT recognizes the need for generating biological reference points such as catch limits and optimal fishing effort (f_{MSY}), among others, in order to manage the fisheries.

Nevertheless, and despite of the economic and social importance of this fishery resource, the normative of this is limited in terms of the specifications required in order to achieve its sustainable use. Certain regulatory guidelines specific for the Gulf of Mexico (e.g., National Fishing Charter (NFC)) are at disposal and used to administer its management [4]. However, the scope of the guidelines is limited given that these do not provide management specifications for the State of Tamaulipas. In part, this is due to the fact that the scientific reports for this fishery resource are scarce. Furthermore, those few reports are mainly focused on capture size analysis rather than management [5–7]. In sum, no scientific research has been made regarding the management of the blue crab in LM as a fishery resource. Particularly for the coast of the State of Tamaulipas, the NFC stablishes that the annual maximum catch limit is 2100 tons per year and that the maximum fishing effort consists of 47 permits, 11,802 hoops, 35,200 traps, and 641 vessels. The NFC also mentions that this fishery is "exploited to its sustainability maximum." This yearly allowed catch limit pertains to an average of the annual catch of the period from 2000 to 2007, and not to the maximum sustainable yield (MSY).

The estimation of the maximum sustainable yield from the surplus production models has been a popular goal in fisheries management even though it has been questioned regarding its supposed equilibrium [8–13]. On the other hand, the conceptualization of this reference point has transitioned from being a target goal into a target limit. Given the overexploitation status of the majority of fisheries in the world, in terms of fisheries management, the fisheries science seeks to minimize the probabilities of exceeding the limit of the MSY (fishery risk) or of the biomass declining beyond the level of natural renewal (stock risk) [14]. With this in mind, in the effort of minimizing probabilities, the precautionary approach in the fisheries management is implicitly included, represented by the fisheries biological reference points (e.g., MSY, maximum sustained effort or E_{MSY}) or those based on the fishing mortality (e.g., F_{MRS}, $F_{0.1}$) [14, 15].

In the context of fisheries management, the line of research regarding the estimation of some fisheries reference points has currently resurfaced in the Middle East, primarily MSY and f_{RMS} [16–24], by means of the adjustment of the Fox, Schaefer, and Pella-Tomlinson models, which are included in some computer packages such as A Surplus-Production Model Incorporating Covariates (ASPIC) [25], which in turn is a stock production model that incorporates the covariance of the parameters. On the other hand, on the topic of model selection, the flow of use of the information criteria (IC) reaching up to multimodel inference, within the framework of the information

theory, arose in parallel. The model selection based on the information theory is a relatively new paradigm in biological sciences and is very much different from the classical method based on the null hypothesis test [26–29].

Therefore, the objective of this research is to estimate fisheries reference points of the blue crab (*Callinectes sapidus*) of the Laguna Madre, Tamaulipas, Mexico, that can be employed to make decisions in the management of this resource.

2. Methods

2.1 Study area: Laguna Madre, Mexico

Laguna Madre is located north of the State of Tamaulipas (23°48′25°30' N y 97° 23′97°52' W) (**Figure 1**). The northern part of the lagoon is delimited by Río Bravo in the municipality of Matamoros and in the southern part by the Soto la Marina River in the municipality of Soto La Marina [30]. Its surface has an area of 2000 km^2, with an average depth of 0.7 m. It is separated from the Gulf of Mexico

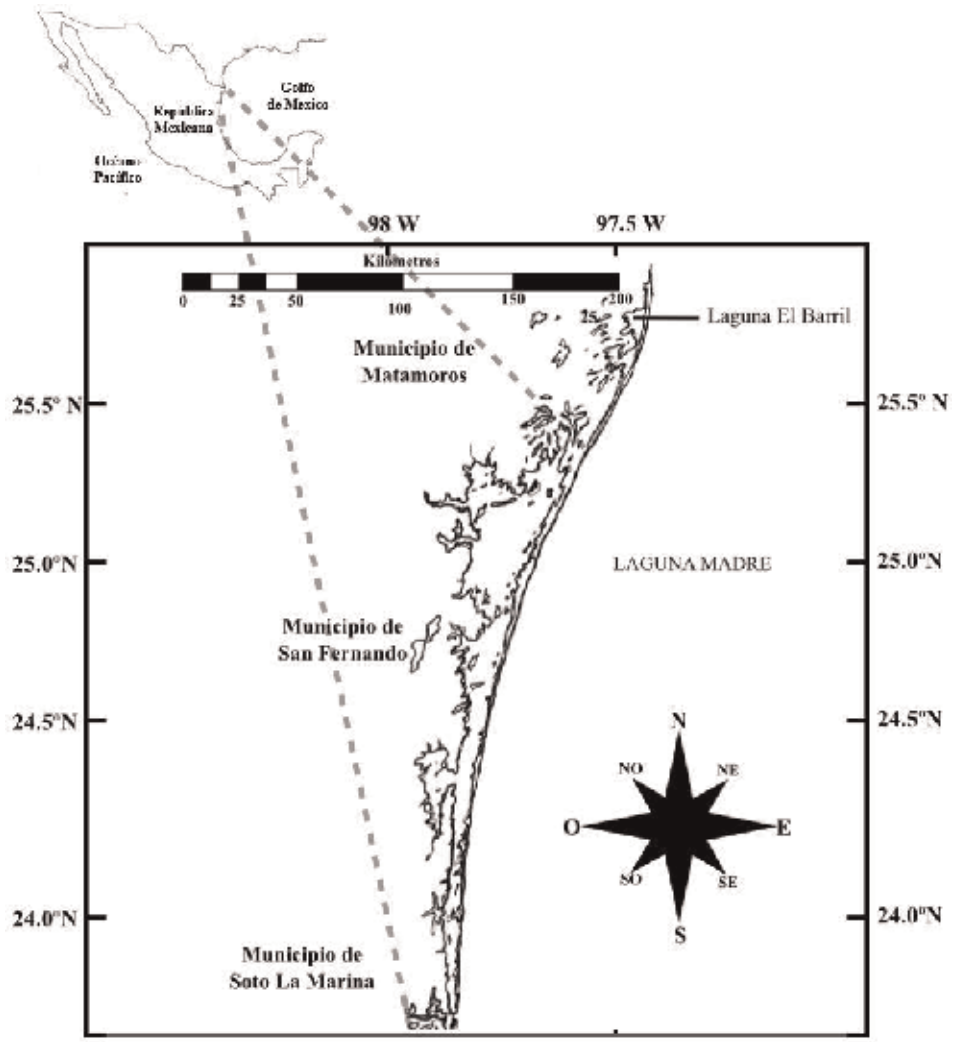

Figure 1.
Laguna Madre, State of Tamaulipas, Mexico.

by a straight and uniform coastal barrier located windward and irregular towards the continental edge. The depression of the lagoon is partially filled by supply of the San Fernando River, thus being divided into two basins: northern and southern [31]. LM has a type BS1 (h') climate which is semiarid with rainfall in the summer though scarce throughout the year, with a winter precipitation between 5 and 10.2% [32]. The surface water of LM has a wide range of salinities, from 21.0 to 51.0 with euryhaline conditions during October (35.0–38.0 psu), poly-euhaline in January (21.0–36.0 psu), and eu-hyperhaline in May and July (33.0–46.0 and 36.0–51.0 psu, respectively) [30].

2.2 Data

The annual historical record of the catch measured in tons of the blue crab and the fishing effort (f), this last one represented by the number of traps (NT), were used for a time series of 14 years, corresponding to the period from 1998 to 2012 (**Table 1**). This information was provided in 2013 by the Fisheries Sub-delegation in Tampico, Tamaulipas, of the delegate of SAGARPA in Tamaulipas. The fishing effort was not standardized for the following reasons: (1) the blue crab fishery in LM is monospecific, (2) the extractive activity of this resource is carried out in a single zone during a single period of the year, and, (3) since the beginning of the fishery, the artisanal fishing fleet has remained technologically stable.

2.3 Models

Both the Schaefer [33] and the Fox [34] models were the two surplus production models (or dynamic biomass models) employed in this study using the following

Years	Catch (tons)	Effort (number of traps)	CPUE
1998	2498	28,500	0.088
1999	2302	45,600	0.05
2000	1103	45,600	0.024
2001	1318	45,600	0.029
2002	1432	25,420	0.056
2003	2699	25,420	0.106
2004	2971	32,600	0.091
2005	3462	32,600	0.106
2006	2140	25,420	0.084
2007	2097	83,450	0.025
2008	2433	92,680	0.026
2009	2362	92,680	0.025
2010	2940	83,450	0.035
2011	2967	73,836	0.04
2012	1927	73,836	0.026

CPUE, Catch per unit effort.

Table 1.
*Catch, fishing effort, and catch per unit effort of the blue crab (*Callinectes sapidus*) in Laguna Madre, México, during the period 1998–2012.*

algorithms: $\frac{dB}{dt} = rB(B_\infty - B)$ [33] and $\frac{dB}{dt} = rB(lnB_\infty - lnB)$ [34], where B is the biomass of the stock, t is the time measured in years, B (K) is the carrying capacity, n is the inclination measure of the curve, and r is the intrinsic rate of population increase. In order to run these models, the ASPIC Version 5.0 computer package [25] was used. This software incorporates the values of the initial proportion, which correspond to the relative catch value of the first year of the time series, concerning the catch of the year with the highest catch value from the same time series. In addition to the variability estimators such as the coefficient of determination (r^2) and the coefficient of variation (CV), the outgoing parameters (management quantities) are the carrying capacity (K), the catchability coefficient (q), the maximum sustainable yield, and the optimal fishing effort (f_{MSY}) (i.e., the maximum number of traps that the body of water can withstand without affecting the stock renewal). The management quantities were obtained by using two types of residual errors: the additive error and the multiplicative error. These types of errors of residual variance were calculated using $\sigma^2, with\ additiveerror = \frac{\sum_{i=1}^{n}\left(y_1 - \widehat{y_1}\right)^2}{n}$, and $\sigma^2, with\ multiplicativeerror = \frac{\sum_{i=1}^{n}\left(\ln\left(\frac{y}{\widehat{y_1}}\right)\right)^2}{n}$, where σ^2 = variance, y_1= observed value, $\widehat{y_1}$ = estimated value, and n = number of data. Also, confidence intervals of the outgoing parameters were estimated at a confidence level of 95% (α = 0.05), according to Sparre and Venema [35]. This was carried out using the following algorithm: $CI = \overline{X} \pm t_{n-1}\left(\frac{\sigma}{\sqrt{n}}\right)$, where CI = confidence interval, t_{n-1} = percentiles of Student's t-distribution, σ = standard deviation, and n = number of data. In this study, t_{n-1}= 2.5, as gathered from the t-distribution table. Given the confidence level of 95%, this percentile was searched in the table and used for the obtaining the t-distribution with $n - 1$ = 14 degrees of freedom.

2.4 Model selection

The information criteria were used for the selection of the model with the best adjustment. These were (a) the Akaike information criterion (AIC) [36], as in the corrected Aikake's information criterion (AICc) [37], given that $n/k < 40$ [26] ($AIC_c = AIC + \frac{2k(k+1)}{n-k-1}$, where $AIC = n^*\hat{\sigma}^2 + 2k$) [39]; (b) the Bayesian information criterion (BIC) ($BIC = -2\hat{\sigma}^2 + kn$) [39]; and (c) the Hannan-Quinn information criterion (HQIC) ($HQIC = -2\hat{\sigma}^2 + 2kn$) [40]. From the shown equations, $\hat{\sigma}^2$ = residual variance, k = number of parameters, and n = number of data.

Once the values of the outgoing parameters and the IC were known, the statistical support was assessed, followed by the quantification of evidence from each model by estimating the differences (Δ_i) and the plausibility (i.e., the weight of the evidence in favor of the model i) of each (w_i), according to the criterion set by Burnham and Anderson [26]. For the estimation of Δ_i, $\Delta i = IC - IC_{min}$ was used, where IC = AICc, BIC, or HQIC and IC_{min} = model with the lowest value of AICc, BIC, or HQIC. According to Burnham and Anderson [26], the scale of Δ_i is described as follows: if $\Delta_i > 10$, it shows that the candidate models lack statistical support and thus should not be taken into account; if $\Delta_i < 2$, the candidate models have high evidence as alternative functions; and if $4 < \Delta_i < 7$, the candidate models can be taken into account, although they count with less statistical support than the previous ones. The w_i were calculated using $w_i = \frac{\exp\left(-\frac{1}{2}\Delta_i\right)}{\sum_{K=1}^{K}\exp\left(-\frac{1}{2}\Delta_i\right)}$, where Δ_i = AICc, BIC, or HQIC difference and K = number of parameters.

3. Results

3.1 Maximum sustained yield according to the initial proportion

The values of K, q, MSY, f_{MSY}, CV, and R^2 of the Fox and the Schaefer models are presented in **Table 2**. Based on the results of the management quantities (B_1/K, K, q, and MSY) of every IP value (from 0.1 to 0.9), the model with the best adjustment was the Fox model, according to r^2, whereas the Schaefer model had the best adjustment, according to the CV (**Table 2**). However, considering the management quantities only for IP = 0.7 and based on the CV (**Table 3**), the regression estimator (r^2), the variability estimators (r^2, CV, σ^2, σ), and IC (AICc, BIC, and HQIC), the selected model was the Fox model (**Table 4**).

Table 3 shows the punctual estimations and the confidence intervals of the management quantities (K, q, MSY, and f_{MSY}) calculated by both models, Fox and Schaefer, according to the type of error in the residual variance and based on the IP = 0.7. Based on the standard deviation, the sizes of confidence intervals are in the following ascending order: Fox (multiplicative), Schaefer (multiplicative), Fox (additive), and Schaefer (additive), respectively. The punctual values of the management measures varied between models, but not between types of error of residual variance.

Model	IP	B_1/K	K	q	MSY	f_{MSY}	CV	r^2
Fox	0.1	0.1337	53,990	0.00008798	2567	146,900	0.3318	0.803
	0.2	0.1337	53,990	0.00008798	2567	146,900	0.3175	0.803
	0.3	0.1337	53,990	0.00008798	2567	146,900	0.342	0.803
	0.4	0.1337	53,990	0.00008798	2567	146,900	0.3166	0.803
	0.5	0.1337	53,990	0.00008798	2567	146,900	0.337	0.803
	0.6	0.1337	54,000	0.00008798	2567	146,900	0.3186	0.803
	0.7	0.1337	54,000	0.00008798	2567	146,900	0.3472	0.803
	0.8	0.1337	53,990	0.00008798	2567	146,900	0.3272	0.803
	0.9	0.1337	54,000	0.00008798	2567	146,900	0.3329	0.803
Logistic (Schaefer)	0.1	1.098	26,850	0.00002528	2006	58,960	0.235	0.517
	0.2	1.098	27,440	0.00002488	2006	58,760	0.205	0.517
	0.3	1.098	27,850	0.00002459	2007	58,610	0.2099	0.516
	0.4	1.098	28,060	0.00002444	2007	58,520	0.2585	0.516
	0.5	1.098	28,200	0.00002435	2008	58,470	0.2079	0.516
	0.6	1.098	28,300	0.00002429	2008	58,430	0.2345	0.516
	0.7	1.098	28,370	0.00002425	2008	58,390	0.2117	0.516
	0.8	1.098	28,420	0.00002422	2009	58,370	0.2273	0.515
	0.9	1.098	28,460	0.0000242	2009	58,350	0.2074	0.515

IP = initial proportion, B1/K = initial biomass divided by carrying capacity, K = carrying capacity, q = catchability coefficient, MSY = maximum sustained yield, f_{MSY} = optimal effort, CV = coefficient of variation, and r^2 = coefficient of determination.

Table 2.
Management quantities (B_1/K, K, q, and MSY) and variability estimators (CV and r^2) according to the Fox and the Schaefer models, in function with the initial proportion of the biomass (IP), of the fishery of the blue crab (Callinectes sapidus) in Laguna Madre, Mexico, during the period of 1998–2012.

Parameters and confidence intervals	Type of error of residual variance			
	Additive		Multiplicative	
	Models		Models	
	Fox	Schaefer	Fox	Schaefer
K	54,000	28,370	54,000	28,370
ILCI (P < 0.05)	53,547	27,559	53,781	28,012
SLCI (P < 0.05)	54,453	29,181	54,219	28,728
q	0.00008798	0.00002425	0.00008798	0.00002425
ILCI (P < 0.05)	0.00008753	0.00002344	0.00008776	0.00002389
SLCI (P < 0.05)	0.00008843	0.00002506	0.00008820	0.00002461
RMS	2567	2008	2567	2008
ILCI (P < 0.05)	2114	1197	2348	1650
SLCI (P < 0.05)	3020	2819	2786	2366
f_{RMS}	146,900	58,390	146,900	58,390
ILCI (P < 0.05)	142,370	57,579	144,710	58,032
SLCI (P < 0.05)	151,430	59,201	149,090	58,748

K = carrying capacity, q = catchability coefficient, MSY = maximum sustained yield, f_{MSY} = optimal fishing effort, ILCI = inferior limit of the confidence interval, and SLCI = superior limit of the confidence interval.
The initial values of the management quantities were estimated using the A Surplus-Production Model Incorporating Covariates (ASPIC) software with 0.7 as the initial proportion, considering the additive and multiplicative errors of the residual variance.

Table 3.
Average values and confidence intervals of the management quantities (K, q, MSY, and f_{msy}) generated by the Fox and logistic (Schaefer) models for the fishery of the blue crab (Callinectes sapidus) in Laguna Madre, Tamaulipas, Mexico.

Selection criteria	Types of error			
	Additive models		Multiplicative models	
	Fox	Schaefer	Fox	Schaefer
r^2	0.813	0.516	0.813	0.516
$\sigma^2_{residual}$	665,217	2,132,349	0.1560	0.4148
σ	816	1460	0.3950	0.6441
k	3	3	3	3
AIC	9,978,258	31,985,238	8.34	12.22
AICc	9,978,256	31,985,235	5.94	9.82
BIC	9,978,297	31,985,277	47.34	51.22
HQIC	9,978,342	31,985,322	92.34	96.22

The initial values of the management quantities were estimated by means of the A Surplus-Production Model Incorporating Covariates (ASPIC) software with 0.7 as the initial proportion, considering the additive and multiplicative errors of the residual variance.

Table 4.
Residual variance (σ^2), standard deviation (σ), number of parameters (k), and the corrected Akaike's information criterion (AICc) as well as the Bayesian (BIC) and the Hannan-Quinn (HQIC) information criteria for each model (Fox and logistic), of the management quantities (K, q, MSY, and f_{MSY}), for the fishery of the blue crab (Callinectes sapidus) in Laguna Madre, Tamaulipas, México.

3.2 Model selection

The values of the parameters r^2, CV, σ^2, σ, and those of the IC (AICc, BIC, and HQIC), which correspond to the Fox and Schaefer models and according to the type of error of residual variance, are presented in **Table 4**. The lowest values of those estimators pertain to the Fox model in both types of errors. Nevertheless, the values of the management measures obtained from this model (Fox) are far from reality, particularly the optimal fishing effort (number of traps).

Figure 2A shows how the fishing catch developed through time as well as the obtained MSY from the models (Schaefer and Fox). It can be observed that, according to the Schaefer model, during the period beginning from 2003 up to 2011, the fishery resource of the blue crab in LM became overexploited. During this period, approximately 24,000 tons of blue crab were captured, with a surplus of 6000 tons as defined by the function based on the difference in tons of catch with the MSY (2008 tons). Regarding the number of traps (f), the f_{MSY} was exceeded from 2007 to 2012 (**Figure 2B**). During this mentioned period (2007–2012), approximately 500,000 traps were registered, with a surplus of 150,000 traps.

As for the Fox model, the results indicate that the resource was overexploited during only 5 (2003, 2004, 2005, 2010, and 2011) out of the 14 years that make up the time series of this study. During these years of overexploitation, the catch should have not exceeded 12,835 tons; but 15,039 tons were gathered instead. This shows a surplus of 2204 tons. In this period of overexploitation, 132, 404, 895, 373, and 400 tons were overfished in the years 2003, 2004, 2005, 2010, and 2011, respectively. This is the equivalent of an overfished resource by 5%, 14%, 26%, 13%, and 13%, respectively. In terms of fishing effort (number of traps), the f_{MSY} was surpassed (14,690 traps) by 40 up to 85% within the whole time series, according to the Fox model.

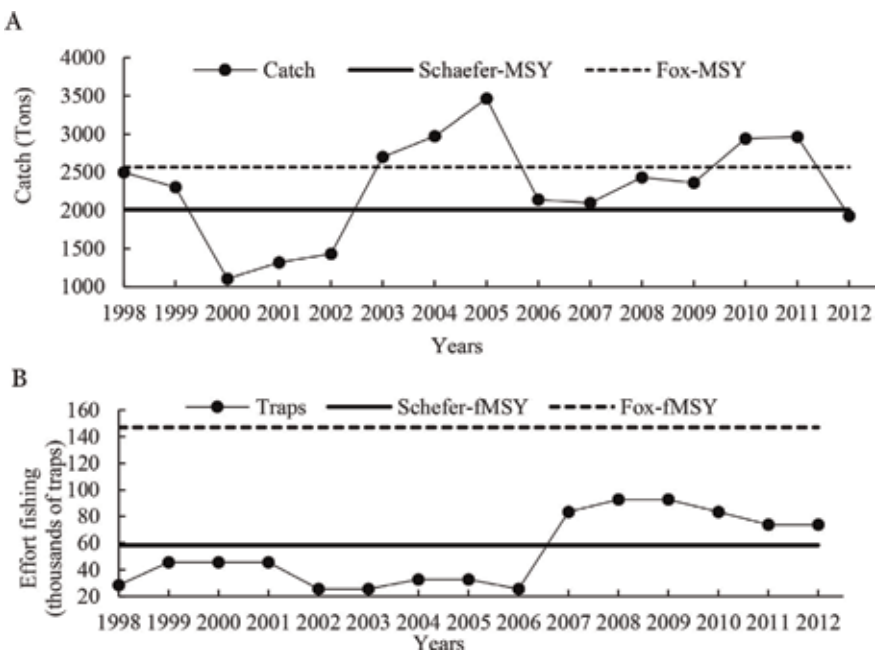

Figure 2.
Fishing catch development through time of blue crab from 1998 to 2012 in Laguna Madre, Tamaulipas, Mexico, with the maximum sustained yield (A) and the number of traps and the optimal number of traps (optimal fishing effort) during this period (B).

4. Discussion

This is the first time that management quantities have been estimated for the fishery of the blue crab (*Callinectes sapidus*) in Laguna Madre, Tamaulipas, Mexico. Furthermore, this is the first work carried out for aquatic organisms on the coast of Tamaulipas, Mexico, in which the information theory is applied with the purpose of selecting models by means of the IC, using the corrected Akaike's information criterion [41] (AICc) (which is used for small samples), the Schwarz or Bayesian information criterion [39] (BIC) and the Hannan-Quinn information criterion (HQIC).

4.1 Management quantities

Fisheries research investigations that deliver fishery management measures through application software programs such as ASPIC and CEDA (catch effort data analysis) to mention some, which include the adjustment of the Fox, Schaefer, and Pella-Tomlinson models, have recently resurfaced [16–24]. However, in this resurgence, there has been an underutilization of the management measures delivered by these software programs given that these only give even more emphasis to the MSY and the f_{MSY}, thus leaving both K and q unused. Both K and q are parameters relative to the initial biomass and the catchability of the fishing gear and, hence, can be used to generate management measures.

The results presented in this study for the two main reference points were MSY = 2567 tons and f_{MSY} = 146,900 traps, from the Fox model, and MSY = 2008 tons and f_{MSY} = 58,390 traps, from the Schaefer model. The model with the best delivered adjustment was the Fox model, according to r^2, CV, and the information criteria. As for the MSY, the Schaefer model presented a result (2008 tons) that was a little more conservative than the one delivered by the Fox model (2567 tons). The difference between both results was of 22%. However, the values of the management measures obtained from this model (Fox model) were far from reality, for the optimal fishing effort (number of traps) particularly. By accepting this model could mean allowing an increase of more than 170% of the fishing effort (number of traps) which in turn could imply an increase of overfishing risk in the short term, whereas, to accept the Schaefer model (f_{RMS} = 58,390), which is a more conservative proposal, could imply the sustainability fortification of this fishery resource. With the Schaefer model, and in relation with the average number of traps from the last 6 years of the time series, the use of about 25 traps (the equivalent of a 30%) would be restricted. This also means a lower social impact without affecting the sustainability of the fishery resource.

4.2 Fishing effort measure

The definition of the fishing effort measure always represents a challenge for fisheries research, given the need for seeking the measure that can best explain the variability of fisheries catch. In this study, the number of traps was used as the measure of fishing effort, assuming that this measure of fishing effort delivers better adjustments to the models than the number of fishermen and vessels can do. Yet, it remains the assessment of the best adjustment of these management measure units for this fishery. A better measure would probably be the time length on which a trap remains underwater; this will also be a pending challenge. The same situation is presented by the shrimp fishery in the Gulf of California, where the approach of using engine power (horsepower) as a measure to normalize fishing power has been

attempted [42]. Nevertheless, it has been considered that this measure does not properly represent the variation of the applied fishing effort since trawls use a speed of 3 knots for efficient fishing [43]. Instead, Morales-Bojórquez et al. [43] suggest that the best measure for this crustacean is the drag time; also, they indicate that the number of vessels is a good measure of fishing effort, which Altran and Loesch suggest as well [44].

The fishing effort (number of traps) in this study is expressed in absolute values and was not normalized by any technique, given that (1) the fishery of the blue crab in Laguna Madre is monospecific, (2) the fishing season is the same throughout LM during the year, and (3) the fishing gear has remained technologically stable since the beginning of the fishery. It is important to properly identify the need for normalizing the fishing effort, or not, given that the results may vary according to the unit of the measure of fishing effort and that the results of some measures could be less realistic. Morales-Bojórquez et al. [43] standardized the number of vessels for the yellowleg or brown shrimp (*Farfantepenaeus californiensis*) fishery in the Gulf of California considering methods of using average efforts. However, the results were not successful [43]. Using average efforts can lead to poor results [45].

4.3 Model selection

In a large number of research publications, the values of the coefficient of determination (r^2) and the coefficient of variation (CV) are established as selection criteria between different candidate models of individual growth [46]. The selection criterion consists of the process of identifying the candidate model with both an r^2 value closest to 1 and the lowest CV. According to Burnham and Anderson [26], r^2 is a measure of the description and variation of the adjustment of the model to fit the data. Regardless of this, it is not a useful criterion to select models that compete to describe the observed data [26]. Because of this, the use of the information criteria is recommended to align with the theory of information [26].

The AICc, BIC, and HQIC have as their foundation the Kullback–Leibler distance, which measures the approximation of the calculated model with the real data; this way, the best candidate model is selected [27, 47]. The information criteria rank the models according to the lower values of these information criteria, so that the models with the lower values of AICc, BIC, and HQIC will be considered as the best models [48–50]. The most important premise on the IC method is to penalize the number of parameters from each model based on the principle of parsimony [46]. In other words, there is a criterion based on the goodness of fit (adjustment) of the model to the data defined by the objective function of maximum likelihood or residual sum of squares (RSS) [46]. At the same time, there is a penalization associated with the total amount of parameters of the model [46].

The NFC establishes that capture over 2100 tons per year should not be allowed. Additionally, it indicates that this fishery is in a state of "exploited to its sustainable maximum." In this study, the model with the best adjustment, according to both the scientific criteria and reality, was the Schaefer model (*MSY* = 2008 tons per year). Following this criterion, the maximum catch recommended by the NFC represents the overexploitation of the blue crab as a fishery resource, given a surplus of 90 tons per year on average.

4.4 Decision criteria

The model selection based on the information theory is a relatively new paradigm in the biological sciences and is very different from the classic method based on null hypothesis testing [26–29]. In this study, the estimators of the coefficient of

determination, standard deviation, and the IC used (AICc, BIC, and HQIC) delivered the same results with respect to the selection of the model with the best adjustment. However, it has been shown that the criteria based on the information theory in the adjustment of the models are those that deliver values with greater certainty for their particular properties according to Burnham and Anderson [26].

5. Conclusions and recommendations

a. Despite the economic and social importance of the crab fishery from Mexico, and in particular the blue crab (*Callinectes sapidus*) on the coast of the Gulf of Mexico, specifically in the Laguna Madre, Tamaulipas, the official measures of fishing management are insufficient and outdated, while those corresponding to unofficial scientists are nonexistent. On the Pacific Ocean side, several species of the genus *Callinectes* concur for which certain regulations (Official Mexican Standard) and planning (Regional Fisheries Management Plan) applicable to fisheries regulation are available. In the specific case of Laguna Madre, Tamaulipas, there is currently no specific regulation or regional fisheries planning; this is the first work that delivers some fisheries management measures such as MSY and f_{MSY}, mainly and specifically for the fishing resource of the blue crab *Callinectes sapidus* in the Laguna Madre, Tamaulipas. Consequently, the comparative analysis of fishery management measures of this species is only carried out between those officially indicated and those thrown by this study, and in particular, these measures are only the MSY and f_{MSY}.

b. The scientific publications on the blue crab *Callinectes sapidus* of the Laguna Madre, Tamaulipas, and the State of Tamaulipas are scarce; the existing ones deal mainly with growth issues and are located in gray literature, with little access. On the contrary, for this same species, in other regions of the Atlantic, such as the Chesapeake Bay, USA, and Lake Maracaibo in Venezuela, there is research on the estimation of fishery management measures, located both in gray literature as in published literature, but infrequent and outdated.

c. According to the results presented in this study, and considering the period analyzed (1998–2012), the fishery resource of the blue crab (*Callinectes sapidus*) of the Laguna Madre, Tamaulipas, was under-exploited for the first 5 years, and subsequently, the last 10 years, an overexploitation is recorded according to the RMS obtained in this same study.

d. It is recommended to use the fishery management measures thrown in this study for blue crab (*Callinectes sapidus*), in particular those corresponding to MSY and f_{MSY} (MSY = 2.008 ton and f_{MSY} = 58.390 traps), specifically for the Laguna Madre, Tamaulipas. It is necessary to incorporate these measures into the applicable regulations in force or add them to the NFC, as well as include them in the Fisheries Management Plan that is being prepared for this purpose. These actions would be in order to contribute to fisheries regulation, and, consequently, to the conservation of the fishery resource.

e. The use of the information criteria (Akaike, Bayesian, and Hannan and Quinn) is proposed to select, according to the best fit, the dynamic biomass models of Schaefer and Fox, since they increase the certainty during the selection process.

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References

[1] Comisión Nacional de Pesca y Acuacultura (CONAPESCA). Anuario Estadístico de Acuacultura y Pesca. México: SAGARPA; 2017. Available at: https://www.conapesca.gob.mx/work/sites/cona/dgppe/2017/ANUARIO_ESTADISTICO_2017.pdf [Last consultation: November 17, 2018]

[2] Rodríguez-Castro JH. Sustentabilidad Pesquera de la Jaiba Azul *Callinectes sapidus* (Rathbun, 1896) en Tamaulipas, México: Un análisis de la Incertidumbre en los parámetros de Crecimiento y en los Puntos de Referencia Para el Manejo de la pesquería [Tesis de Doctorado]. Ciudad Victoria, Tamaulipas: Universidad Autónoma de Tamaulipas; 2017

[3] Secretaria de Medio Ambiente y Recursos Naturales (SEMARNAT). Acuerdo por el que se da a conocer el Resumen del Programa de Manejo del Área de Protección de Flora y Fauna Laguna Madre y Delta del Río Bravo. México, D.F.: Diario Oficial de la Federación. DOF; 2015

[4] Diario Oficial de la Federación (DOF). del 25 de agosto del 2006. Carta Nacional Pesquera. SAGARPA, CONAPESCA. 2006. Segunda Sección. Gobierno Constitucional de los Estados Unidos Mexicanos México; 2006

[5] Medellín-Avila M, Arzate-Aguilar E, Gómez-Ortíz MA, González-Cruz A. La pesquería ribereña de la jaiba (*Callinectes sapidus*) en la Laguna, Madre, Tam., durante 2001 y 2002. In: Memorias del II Foro Científico de Pesca Ribereña. 20–22 de Octubre del 2003; Ciudad de Colima, Col. 93–94. 2003. Retrieved from http://www.inapesca.gob.mx/portal/documentos/publicaciones/14II+foro+pesca+riberena2003.pdf

[6] Leo-Peredo AS, Conde-Galaviz E. Estudio pesquero de la jaiba (*Callinectes sapidus*) en la región norte de la Laguna Madre, Tamaulipas. In: Memorias del III Foro Científico de Pesca Ribereña. 3–5 de Octubre del 2006. Trabajo 075: Cartel. Puerto Vallarta, Jal. 2006. pp. 153-154. Retrieved from http://www.inapesca.gob.mx/portal/documentos/publicaciones/15III%20foro%20pesca%20riberena2006.pdf

[7] Leo-Peredo AS, Conde-Galaviz E. Algunos aspectos de la pesquería de la jaiba azul (*Callinectes sapidus*) en la parte norte de la Laguna Madre, Tamaulipas. In: Memorias del IV Foro Científico de Pesca Ribereña. 9–11 de Septiembre 2008. Trabajo 046: Cartel. Acapulco, Gro. 2008. pp. 153-154. Retrieved from http://www.inapesca.gob.mx/portal/documentos/publicaciones/16%20memoria+IV+foro+pesca+riber e%C3%B1a-2008.pdf

[8] Ricker WE. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada. 1975;**191**:382

[9] Pitcher TJ, Hart PJ. Fisheries Ecology. London: Chapman and Hall; 1982

[10] Hilborn R, Walters CJ. Quantitative Fisheries Stock Assessment: Choice, Dynamics and Uncertainty. New York: Chapman and Hall; 1992. 570 pp.

[11] Prager MH. A suite of extensions to a nonequilibrium surplusproduction model. Fishery Bulletin. 1994;**92**: 374-389

[12] Quinn TJ II, Deriso RB. Quantitative Fish Dynamics. New York, USA: Oxford University Press; 1992. 542 pp

[13] Maunder MN, Sibert JR, Fonteneau A, Hampton J, Kleiber P, Harley SJ. Interpreting catch per unit

effort data to assess the status of individual stocks and communities. ICES Journal of Marine Science: Journal du Conseil. 2006;**63**(8):1373-1385

[14] Mace PM. Relationships between common biological reference points used as thresholds and targets of fisheries management strategies. Canadian Journal of Fisheries and Aquatic Sciences. 1994;**51**(1):110-122

[15] FAO. Reference points for fishery management: Their potential application to straddling and highly migratory resources. In: FAO Fisheries Circular 864. Food and Agriculture Organization of the United Nations; 1993. 52 pp.

[16] Panhwar SK, Liu Q, Khan F, Siddiqui PJ. Maximum sustainable yield estimates of Ladypees, *Sillago sihama* (Forsskål), fishery in Pakistan using the ASPIC and CEDA packages. Journal of Ocean University of China. 2012;**11**(1): 93-98

[17] Panhwar SK, Liu Q, Khan F, Waryani B. Maximum sustainable yield estimates of spiny lobster fishery in Pakistan using non-equilibrium CEDA package. Russian Journal of Marine Biology. 2012;**38**(6):448-453. DOI: 10.1134/S1063074012060077

[18] Kalhoro MA, Liu Q, Memon KH, Chang MS, Jatt AN. Estimation of maximum sustainable yield of Bombay Duck, *Harpadon nehereus* fishery in Pakistan using the CEDA and ASPIC packages. Pakistan Journal Zoology. 2013;**45**(6):1757-1764

[19] Kalhoro MA, Liu Q, Memon KH, Waryani B, Soomro SH. Maximum sustainable yield of Greater lizardfish *Saurida tumbil* fishery in Pakistan using the CEDA and ASPIC packages. Acta Oceanologica Sinica. 2015;**34**(2):68-73. DOI: 10.1007/s13131-014-0463-0

[20] Siyal FK, Li Y, Tianxiang G, Liu Q. Maximum sustainable yield estimates of silver pomfret, *Pampus argenteus* (family: Strometidae) fishery in Pakistan. Pakistan Journal of Zoology. 2013;**45**:447-452

[21] Wang Y, Liu Q. Application of CEDA and ASPIC computer packages to the hairtail (*Trichiurus japonicus*) fishery in the East China Sea. Chinese Journal of Oceanology and Limnology. 2013;**31**(1): 92-96

[22] Memon KH, Liu Q, Kalhoro MA, Nabi A, Kui Z. Maximum sustainable yield estimates of barramundi *Lates calcarifer* fishery from Pakistani waters. Indian Journal of Geo-Marine Sciences. 2015;**44**(6):825-832

[23] Liao B, Liu Q, Zhang K, Wang X. Verifier of the maximum sustainable yield estimates of the southern Atlantic albacore, *Thunnus alalunga*, stock. Fisheries Management and Ecology. 2016;**23**(2):161-168

[24] Liao B, Liu Q, Zhang K, Baset A, Memon AM, Memon KH, et al. A continuous time delay-difference type model (CTDDM) applied to stock assessment of the southern Atlantic albacore and *Thunnus alalunga*. Chinese Journal of Oceanology and Limnology. 2016;**34**(5):977-984

[25] Prager MH. A Stock-Production Model Incorporating Covariates (version 5) and auxiliary programs. Beaufort Laboratory Document. CCFHR (NOAA) Miami Laboratory Document MIA-92/93–55, 2005. BL-2004-01

[26] Burnham KP, Anderson DR. Model Selection and Multimodel Inference: A Practical Information Theoretic Approach. 2nd ed. New York: Springer; 2002. 488 p

[27] Katsanevakis S. Modelling fish growth: Model selection, multi-model inference and model selection uncertainty. Fisheries Research. 2006;**81**(2–3):229-235

[28] Beninger PG, Boldina I, Katsanevakis S. Strengthening statistical usage in marine ecology. Journal of Experimental Marine Biology and Ecology. 2012;**426-427**:97-108

[29] Katsanevakis S. Multi-model inference and model selection in Mexican fisheries. Ciencia Pesquera. 2014;**22**(1):6-7

[30] Ocaña-Luna A, Hernández-Batún G, Sánchez-Ramírez M. Abundancia y distribución de juveniles de *Farfantepenaeus aztecus* (Ives 1891), F. *duorarum* (Burkenroad 1939) y *Litopenaeus setiferus* (Linnaeus 1767) en la Laguna Madre, Tamaulipas, México. Hidrobiológica. 2008;**18**(3):199-208

[31] Yánez A, Schlaepfer CJ. Composición y distribución de los sedimentos recientes de la Laguna Madre, Tamaulipas. Boletín del Instituto de Geología. UNAM. 1968;**84**:5-34

[32] Secretaría de Programación y Presupuesto (SPP). Atlas Nacional del Medio Físico. México. 1981. pp. 80–97

[33] Schaefer M. Some aspects of the dynamics of populations important to the management of the commercial marine fisheries. Bulletin Inter-American Tropical Tuna Commission. 1954;**1**(2):23-56

[34] Fox WW. An exponential surplus yield model for optimizing exploited fish populations. Transactions American Fisheries Society. 1970;**99**:80-88

[35] Sparre P, Venema S. Introducción a la evaluación de recursos pesqueros tropicales. In: Parte 1. Manual. Valparaíso: Food and Agriculture Organization of the United Nations FAO; 1995. 420 pp

[36] Akaike H. Maximum likelihood identification of Gaussian autoregressive moving average models. Biometrika. 1973;**60**(2):255-265

[37] Sugiura N. Further analysis of the data by Akaike's information criterion and the finite corrections. Communications in Statistics, Theory and Methods. 1978;**A7**:13-26. DOI: 10.1080/03610927808827599

[38] Burnham KP, Anderson DR. Multimodel inference understanding AIC and BIC in model selection. Sociological Methods and Research. 2004;**33**(2):261-304

[39] Schwarz G. Estimating the dimension of a model. Annals of Statistics. 1978;**6**:461-464

[40] Hannan EJ, Quinn BG. The determination of the order of an autoregression. Journal of the Royal Statistical Society. 1979;**41B**:190-195

[41] Akaike H. A new look at the statistical model identification. IEEE Transactions on Automatic Control. 1974;**19**(6):716-723

[42] Díaz de Leon-Corral JA. Exploitation and Management of the Shrimp Fishery from Sinaloa, México [PhD dissertation]. London: Imperial College; 1993. 256 p

[43] Morales-Bojórquez E, López-Martínez J, Hernández-Vázquez S. Modelo dinámico de captura y esfuerzo para el camarón café *Farfantepenaeus californiensis* (Holmes) del Golfo de California, México. Ciencias Marinas. 2001;**27**(1):105-124

[44] Altran SM, Loesch JG. An analysis of weekly fluctuations in catchability coefficients. Fishery Bulletin. 1995;**93**: 562-567

[45] Polacheck T, Hilborn R, Punt AE. Fitting surplus production models: Comparing methods and measuring uncertainty. Canadian Journal of Fisheries and Aquatic Sciences. 1993; **50**(12):2597-2607

[46] Guzmán-Castellanos AB, Morales-Bojórquez E, Balart EF. Estimación del crecimiento individual en elasmobranquios: la inferencia con modelos múltiples. Hidrobiológica. 2014;**24**(2):137-150

[47] Katsanevakis S, Maravelias CD. Modelling fish growth: Multi-model inference as a better alternative to a priori using von Bertalanffy equation. Fish and Fisheries. 2008;**9**(2):178-187

[48] Cailliet GM, Smith WD, Mollet HF, Goldman KJ. Age and growth studies of chondrichthyan fishes: The need for consistency in terminology, verification, validation, and growth function fitting. Environmental Biology of Fishes. 2006; **77**:211-228

[49] Romine JG, Grübbs RD, Müsick JA. Age and growth of the sandbar shark, *Carcharhinus plumbeus*, in Hawaiian waters through vertebral analysis. Environmental Biology of Fishes. 2006; **77**:229-239

[50] Griffiths SP, Fry GC, Manson FJ, Loü DC. Age and growth of long tail tuna (*Thunnus tonggol*) in tropical and temperate waters of the central indo-Pacific. International Council for the Exploration of the Sea. Journal of Marine Science. 2010;**67**(1):125-134

Section 3

Genetics

Chapter 8

A Comparison of Genetic Variation in Two Endemic Thermal Spring Isopods, *Thermosphaeroma thermophilum* and *T. milleri* (Crustacea - Isopoda: Sphaeromatidae)

Veijo Jormalainen, James C. Boothroyd, Autumn Dove and Stephen M. Shuster

Abstract

Populations with reduced gene flow and restricted population size are expected to show reduced genetic variation. Using starch gel electrophoresis, we examined allozyme variation at 12 loci in two species of freshwater, sphaeromatid isopods. *Thermosphaeroma thermophilum*, an endangered species, inhabits a single thermal spring in central New Mexico, USA; and *T. milleri*, inhabits a more complex thermal spring system in northern Chihuahua, México. We found no significant differences in allelic variation between the sexes within each species. Between species, electromorphs at each locus differed significantly in both number and moiety on the gel, with *T. milleri* showing greater polymorphism and greater heterozygosity than *T. thermophilum*. Nei's unbiased genetic distance, calculated using the nine loci common to both populations (D = 0.75), was consistent with morphological classification of *T. thermophilum* and *T. milleri* as separate species, as well as with molecular analyses suggesting that these populations have been separated since the late Cretaceous (88 myr). Moreover, consistent with the theoretical expectation that small, isolated populations will exhibit reduced genetic variation, *T. thermophilum*, an endangered species, exhibited significantly less genetic variation than the more numerous and less confined *T. milleri*. We compare our results with other recent studies using this approach to understand the population genetics of natural populations.

Keywords: allozymes, endangered species, Mexico, New Mexico, Socorro isopod

1. Introduction

Wright [1] observed that restricted gene flow and reduced population size can lead to population differentiation and ultimately to speciation. Genetic isolation by distance as well as speciation occurring in the presence of geographic barriers (i.e., allopatric speciation) are well-documented phenomena in a range of taxa [2–4].

Genetic variation is expected to decrease with decreased population size due to fixation of alleles. Frankham [5] empirically demonstrated this effect by comparing genetic variation of island populations and their mainland counterparts. A significant majority of island populations showed reduced genetic variation (average reduction in heterozygosity = 29%, N = 202 comparisons). Moreover, insular endemic species showed significantly greater reductions in genetic variation compared to non-endemic populations [6]. Reduced genetic variation has a strong correlation with reduced population fitness [6]. Species that occupy particular freshwater habitats often have limited abilities to disperse to alternative environments [7, 8]. Freshwater endemic populations are therefore extremely vulnerable to extinction, and a detailed understanding of their genetic diversity is valuable to conservation efforts [6].

With increasing sophistication of methods for assessing genetic variation and documenting parentage using DNA sequence data [9], starch gel electrophoresis has been displaced in favor of these demonstrably more precise methods. Nevertheless, allozyme analysis retains certain advantages over molecular methods; it is cheaper, involves fewer procedural steps, seldom requires optimization, and requires no exploratory studies to identify primers [10]. Most importantly, genetic differences identified by using allozymes provide a clear and conservative method for distinguishing populations. Thus, while no longer fashionable, starch gel electrophoresis remains a useful approach for estimating genetic variation within and among species. Recent studies confirm the continued value of the starch gel and other protein electrophoresis methods for investigating population differentiation across a broad range of taxa, including plants [11–14], invertebrates [15–17] and vertebrates [18–20].

The sphaeromatid isopod genus, *Thermosphaeroma*, consists of seven known species, each endemic to a single, thermal, freshwater habitat in southwestern North America. Monophyly within this genus is unambiguous [21], thus *Thermosphaeroma* provides an excellent system for genetic and evolutionary comparisons among species. In this paper, we report genetic variation in two species of *Thermosphaeroma*; *T. thermophilum*, the endangered Socorro isopod [22, 23] is endemic to a single thermal spring near Socorro, New Mexico, USA. Its congener, *T. milleri*, is also an endemic species, but inhabits a larger and spatially more complex spring system located west of Villa Ahumada, Chihuahua, México, and has a greater estimated population size (Shuster, unpubl. data). Due to the large geographic distance separating these two endemic species (over 500 km), and thus the extreme unlikelihood of gene flow between populations, we expected genetic differentiation between these species to be considerable, despite their close taxonomic relationship [21]. We also expected genetic variation to be significantly reduced in *T. thermophilum* compared to *T. milleri*, given the above predictions of population genetic theory as well as with the Socorro isopod's status as an endangered species [24, 25]. As this is the first detailed description of allozyme variation in this genus, we provide descriptions of electromorphs at each locus. We know of no studies comparing genetic variation between closely related, endemic species with such marked differences in habitat and population size.

2. Materials and methods

2.1 Sampling and processing of isopods

T. thermophilum: Sedillo Spring, the natural habitat of *T. thermophilum*, was modified in the early 1900s to supply water to a thermal spring bathhouse (Evergreen) as well as to the city of Socorro, New Mexico [26]. The bathhouse is now abandoned,

and the species is confined to the concrete pools and gutters through which the remaining surface waters of the spring flow. More detailed descriptions of this restricted habitat and estimates of population size (<3000 individuals) are provided in Federal Register [22] and Shuster [23–25, 27–30].

Using a fine mesh net, we collected samples of approximately 100 *T. thermophilum* from the substrate and walls of pool 2 [23] in November 1993 and in June 1995. In 1993, live isopods were placed in insulated containers with spring water and transported to Flagstaff, AZ, where 13 adult males and 11 adult females from the original sample were placed on ice for 10 min, euthanized by removing the cephalon with sharp forceps, and divided into five tissue sub-samples (cephalon, upper pereon, lower pereon, pereopods and pleotelson). Each tissue sub-sample was placed into a well in one of five separate ELISA dishes with 25 l 0.05 M Tris-HCl buffer and frozen at −80°C until samples were electrophoresed. In 1995, isopods were transported to Flagstaff for use in behavioral experiments [28–30]. Tissue samples were collected from live specimens by placing individuals on ice for 10 min and then amputating and freezing the left or right 7th pereopod.

T. milleri: Several thermal springs exist on Ejido Rancho Nuevo, located west of Villa Ahumada in northern Chihuahua, México. The largest spring, Ojo Caliente, arises from at least five sources within a 200 m^2 area beneath a large cottonwood tree and flows SSW to form a 3–5 m wide stream, which is diverted to supply water to Ejido crops and livestock. Mosquitofish, pupfish, crawfish and large planarians all inhabit the stream whose gravel and mud banks are well-covered with grass and other aquatic vegetation. The population size is estimated to exceed 1.5×10^5 individuals (Shuster, unpubl. data). More details of this habitat are provided in Bowman [31] and Davis [21].

Using a fine mesh net, we collected approximately 100 *T. milleri* from the substrate and vegetation of Ojo Caliente in August 1994 and in December 1995. The 1994 sample was processed as described above for *T. thermophilum*. The 1995 sample was maintained in laboratory aquaria for use in behavioral experiments [28–30]. Tissue samples were collected from live specimens by placing individuals on ice for 10 min and then removing the left or right seventh pereopod with fine forceps. Each pereopod was frozen as described above until samples were electrophoresed.

2.2 Electrophoresis

Frozen tissue samples were thawed on crushed ice, ground with an additional 30 ml 0.05 M Tris-HCl pH 7.5 buffer using a glass rod, and loaded onto 12% starch gels using buffer systems described in Sassaman [32]. Gels were run at 35 mA for 4 h, sliced and stained for 12 enzyme loci (see below). Electrophoretic signals were identified by measuring the height of each signal's leading edge on the gel above its sample well on the gel, and were scored among the two *Thermosphaeroma* species by identifying the fastest running electromorph as "1" and slower electromorphs as 2–3. To verify the relative position of electromorphs on gels between species after all individuals were initially scored, we reran samples from individuals who exhibited the range of allelic variation for both species on the same gels.

For each locus and within each sex, sample and species, we identified three measures of allelic diversity, the polymorphic index [PI = $1 - (\Sigma p_i^2)$, where p_i = the population frequency of each allele, i], the effective number of alleles [ENA = $1/(\Sigma p_i^2)$], and observed heterozygosity (H). We also performed goodness of fit G-tests for deviations from Hardy-Weinberg (hereafter HW) expectations [4]. We calculated the average and standard error of the first three measures and compared PI, ENA and H across loci using U-tests, first between sexes within species using all samples collected, then between species with the sexes pooled. Because of the large

geographic distance separating these two species (>500 km), we did not estimate Wright's F-statistics, assuming that gene flow between these morphologically distinct populations was negligible. Instead we calculated [33] unbiased genetic distance (D) using used Miller's [34] TFPGA which provides a weighted average estimate of D across all loci. We assumed the inbreeding coefficient within each collection equaled F = (1—H) [4].

3. Results

3.1 Electrophoretic signals

The two *Thermosphaeroma* species exhibited two groups of electrophoretic signals, those in which alleles appeared to be similar in moiety on the gel between species (that is, electromorphs appears to reside at similar positions with respect to one another on the gel), and those in which alleles appeared distinct in both moiety and character. We observed apparently similar electromorphs between the species at *Pgm*1, *Pgi*, *Me*, *Mdh*1, *Mdh*2 and 6*pgdh*. Distinct electrophoretic signals appeared at *Pgm*2, *Got*, *Xdh*, *Idh*1, *Idh*2 and *Hex*.

In *T. thermophilum*, phosphoglugomutase loci (*Pgm*1 and *Pgm*2) were monomorphic, visible as tight bands, located at 31.7 and 28.8 mm, respectively, above sample wells. In *T. milleri*, only *Pgm*1 was visible. This locus also appeared as a tight band but exhibited three alleles. $Pgm1^1$ appeared identical in both species, with alleles $Pgm1^2$ and $Pgm1^3$ in *T. milleri* visible at 27.1 and 22.7 mm, respectively, above sample wells.

Phosphoglucose isomerase (*Pgi*) appeared equivalent in moiety in both species, with signals visible as tight bands with small amounts of trailing anodic signal. Two alleles were identifiable, with Pgi^1 located 21.6 mm, and Pgi^2 located 18.9 mm, above sample wells. In both species, heterozygotes appeared as trimers, suggesting that the functional *Pgi* molecule consists of two units which combine at random [35].

Both species shared a similar fast signal at malic enzyme (Me^1), located at 20.2 mm above sample wells; *T. thermophilum* was monomorphic for this allele, whereas *T. milleri* showed two additional loci, Me^2 at 16.4 mm and Me^3 at 14.9 mm above sample wells. All signals in both species appeared as tight, dense bands. At 6-phosphoglugose dehydrogenase (6*pgdh*), the fast electromorph (6-$pgdh^1$) and the intermediate electromorph (6-$pgdh^2$) appeared similar for both species at 37.9 and 32.5 mm above sample wells, respectively. *T. milleri* showed a third electromorph (6-$pgdh^3$) at 29.8 mm, which was not observed in *T. thermophilum*.

Malic dehydrogenase 1 (*Mdh*1) has been recognized as a mitochondrial signal in certain crustaceans (C. Sassaman, pers. com.), thus recognition of alleles at this locus is questionable. However, we identified similar fast signals in both species ("$Mdh1^1$"), appearing as broad bands at 35.5 mm above sample wells, as well as a slower signal ("$Mdh1^2$") in *T. milleri* appearing at 30.4 mm above sample wells. Although we did not include this locus in HW analyses or in calculations of D, we have provided estimates of PI and ENA to illustrate the diversity in these signals within each species. The fast electromorph at *Mdh*2 reached similar locations above sample wells in both species ($Mdh2^1$ at 10.7 mm). However, signal density differed such that in the monomorphic *T. thermophilum* $Mdh2^1$ signal was clearer and more narrow, whereas in *T. milleri* signals were polymorphic ($Mdh2^2$ at 5.1 mm), less dense and showed considerable trailing anodic signal.

The species were distinct at glutamate oxaloacetate transaminase (*Got*). A single allele was visible in *T. thermophilum* as a tight band 38.7 mm (Got^1) above sample wells. In *T. milleri*, two alleles were visible as somewhat broader bands at 29.4 mm (Got^1) and 24.9 mm (Got^2) above sample wells. The species were distinct at xantine

dehydrogenase (*Xdh*) as well. Both species showed broad, monomorphic signals; *T. thermophilum* at 12.0 mm (Xdh^1) and *T. milleri* at 18.3 mm (Xdh^2). Species differences were also clear at hexokinase (*Hex*), both species showing broad and variable, but distinct signals. Two alleles were visible in *T. thermophilum*, Hex^1 at 33.4 mm and Hex^2 at 24.6 mm, and three alleles were visible in *T. milleri*, Hex^3 at 23.2 mm, Hex^4 at 19.8 mm and Hex^5 at 12.8 mm.

3.2 Genetic differences between species

Allele frequencies at all loci in both species conformed to Hardy-Weinberg equilibrium (**Table 1**), suggesting that Mendelian inheritance and selective neutrality can be assumed for these loci [36]. We found no significant differences in polymorphic indices (PI), effective numbers of alleles (ENA) or heterozygosity (H) between the sexes within either species (U-tests: *T. thermophilum*: PI (mean ± SE, n = 17): males, 0.06 ± 0.04; females, 0.08 ± 0.05; $z = -0.22$, $P = 0.83$, NS; ENA: males, 1.09 ± 0.07; females, 1.13 ± 0.09; $z = -0.22$, $P = 0.83$, NS; H: males, 0.05 ± 0.03; females, 0.22 ± 0.14; $z = -0.18$, $P = 0.86$, NS; *T. milleri*: PI (mean ± SE, n = 10): males, 0.20 ± 0.06; females, 0.29 ± 0.10; $z = -0.31$, $P = 0.76$, NS; ENA: males, 1.33 ± 0.12; females, 1.23 ± 0.19; $z = -0.38$, $P = 0.70$, NS; H: males, 0.22 ± 0.06; females, 0.30 ± 0.13; $z = -0.19$, $P = 0.85$, NS). Therefore, the sexes were combined for further analysis.

Locus	Species	Sample	Sex	N	EMs	PI	ENA	H	HW	P
PGM1	*thermophilum*	11/93	M	13	1	0.00	1.00	0.00	—	—
			F	12	1	0.00	1.00	0.00	—	—
		6/94	M	100	1	0.00	1.00	0.00	—	—
			F	14	1	0.00	1.00	0.00	—	—
	milleri	12/95	M	103	3	0.50	1.99	0.52	0.28	N.S.
PGM2	*thermophilum*	11/93	M	13	1	0.00	1.00	0.00	—	—
			F	12	1	0.00	1.00	0.00	—	—
		6/94	M	100	1	0.00	1.00	0.00	—	—
			F	14	1	0.00	1.00	0.00	—	—
	milleri	12/95	No signal							
GOT	*thermophilum*	11/93	M	13	1	0.00	1.00	0.00	—	—
			F	12	1	0.00	1.00	0.00	—	—
	milleri	12/95	M	13	2	0.14	1.17	0.15	0.09	N.S.
			F	12	1	0.00	1.00	0.00	—	—
PGI	*thermphilum*	11/93	M	13	1	0.00	1.00	0.00	—	—
			F	12	1	0.00	1.00	0.00	—	—
		6/94	M	100	2	0.02	1.02	0.00	0.01	N.S.
			F	14	1	0.00	1.00	0.00	—	—
	milleri	12/95	M	103	3	0.25	1.34	0.25	0.52	N.S.
			F	12	2	0.08	1.09	0.08	0.02	N.S.
XDH	*thermophilum*	11/93	M	13	1	0.00	1.00	0.00	—	—
			F	12	1	0.00	1.00	0.00	—	—
	milleri	12/95	M	4	1	0.00	1.00	0.00	—	—
			F	6	1	0.00	1.00	0.00	—	—
ME	*thermophilum*	11/93	M	13	1	0.00	1.00	0.00	—	—

Locus	Species	Sample	Sex	N	EMs	PI	ENA	H	HW	P
			F	12	1	0.00	1.00	0.00	—	—
		6/94	M	75	1	0.00	1.00	0.00	—	—
			F	5	1	0.00	1.00	0.00	—	—
	milleri	12/95	M	35	3	0.27	1.37	0.31	1.22	N.S.
			F	12	2	0.15	1.18	0.20	0.10	N.S.
IDH1	*thermophilum*	11/93	M	9	1	0.00	1.00	0.00	—	—
			F	5	1	0.00	1.00	0.00	—	—
	milleri	12/95	M	13	1	0.00	1.00	0.00	—	—
IDH2	*thermophilum*	11/93	M	13	1	0.00	1.00	0.00	—	—
			F	12	1	0.00	1.00	0.00	—	—
	milleri	12/95	No signal							
MDH1	*thermophilum*	11/93	M	13	1	0.00	1.00	0.00	—	—
			F	12	1	0.00	1.00	0.00	—	—
		6/96	M	85	2	0.12	1.14	0.13	0.41	N.S.
			F	9	2	0.20	1.24	0.22	0.14	N.S.
	milleri	12/95	M	102	2	0.13	1.15	0.14	0.55	N.S.
			F	11	1	0.00	1.00	0.00	—	—
MDH2	*thermophilum*	11/93	No signal							
	milleri	12/95	M	103	2	0.07	1.08	0.08	0.17	N.S.
			F	11	1	0.00	1.00	0.00	—	—
HEX1	*thermophilum*	11/93	M	13	2	0.43	1.75	0.31	1.00	N.S.
			F	8	2	0.50	2.00	0.75	2.00	N.S.
	milleri	12/95	M	13	3	0.50	2.00	0.55	3.57	N.S.
			F	4	2	0.43	1.75	0.43	0.29	N.S.
6PGDH	*thermophilum*	11/93	M	13	1	0.00	1.00	0.00	—	—
			F	12	1	0.00	1.00	0.00	—	—
		6/96	M	48	2	0.06	1.06	0.06	0.05	N.S.
			F	17	2	0.13	1.15	1.43	0.04	N.S.
	milleri	12/95	M	100	3	0.14	1.16	0.15	0.66	N.S.
			F	11	3	0.24	1.32	0.27	0.27	N.S.

Number of electrophoretic morphs (EMs), polymorphic index (PI), effective number of alleles (ENA), observed heterozygosity (H), results of G-test for deviation from the Hardy-Weinberg equilibrium (HW), and significance of deviation (P).

Table 1.
Electrophoretic variation in Thermosphaeroma thermophilum and T. milleri.

Between species, each of the three estimators of genetic variation differed significantly (U-tests, $n = 54$: PI: *T. thermophilum*: 0.07 ± 0.03, $n = 34$; *T. milleri*: 0.25 ± 0.06, $n = 20$; $z = -3.08$, $P = 0.002$; ENA: *T. thermophilum*: 1.11 ± 0.06, $n = 34$; *T. milleri*: 1.28 ± 0.11, $n = 20$; $z = -2.48$, $P = 0.01$; H: *T. thermophilum*: 0.13 ± 0.07, $n = 34$; *T. milleri*: 0.26 ± 0.07, $n = 20$; $z = -2.55$, $P = 0.01$). Nei's [33] unbiased genetic distance, D, equaled 0.75, a value consistent with species-level genetic differences in a number of species [33].

4. Discussion

Strong, directional selection could explain reduced genetic variation in *T. thermophilum* compared to *T. milleri*, although we found no significant deviation from HW expectations at the four variable loci (**Table 1**) in *T. thermophilum*, and no deviations from HW expectations at any locus examined in *T. milleri*. The *T. thermophilum* population is reported to have undergone a severe bottleneck in the mid-1980s, when a valve controlling the water supply to Sedillo Spring was destroyed by vandals (B. Lang, pers. comm.). This event, as well as past destruction of the natural habitat by private and municipal water projects may also have reduced the available habitat, reduced population size, and thus reduced genetic variability in the *T. thermophilum* population. This explanation is consistent with average heterozygosity (H) in *T. thermophilum* equaling half of that observed in *T. milleri*.

However, these factors do not explain why some loci (*Hex*[1]; 6-*pgdh*) in *T. thermophilum* have remained highly variable. Recent bottlenecks in other species appear to reduce genetic variation in all loci simultaneously [37, 38] and reductions in allelic diversity may persist for millennia [39]. Selection can maintain polymorphism in finite populations [4]. However, in Sedillo Spring, the source of selection and the isopod characteristics on which it may act, are unknown. Thus, the existence of genetic variation in some but not all loci in the endangered Socorro isopod population, while encouraging from a species management perspective, for now remains unexplained.

Genetic population structure is well-documented in isopod crustaceans using a variety of genetic markers. Allozymes are most commonly used to identify genetic differences within and among populations [36, 40–43] although pigmentation patterns known to exhibit Mendelian inheritance have been and are still widely used in documenting population differences [44–49]. Molecular markers are increasingly used to document genetic differences among isopod populations, and preliminary data using mitochondrial DNA sequences have been used successfully to distinguish congeners within the genus *Thermosphaeroma* [22].

Although molecular genetic methods do provide more precise information on genetic differences within and among populations, protein electrophoresis remains a useful tool for investigating population differences. Because allozyme loci are usually codominant, clear indications of allelic differentiation, isolation by distance and most standard measures of genetic diversity can all be identified without approximation [12–14, 16]. Population differences detected using this approach are certain to be more conservative than molecular analyses [9]. Moreover, despite continued reduction of the cost of molecular analyses, electrophoresis is remains a cheaper and more sample efficient method—particularly for large samples—than most analyses using DNA.

Our results using allozyme variation, indicate that *Thermosphaeroma milleri* and *T. thermophilum* are genetically distinct at the level of separate species, a result consistent with previous morphological [49] and molecular genetic analyses [22]. Our main finding, that genetic variation in *T. thermophilum* is sharply reduced compared to its closest relative, is consistent with population genetic theory [1, 3, 4] and with recent empirical analyses [5] indicating that reduced population size reduces genetic variation within populations, specifically within the *T. thermophilum* population.

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References

[1] Wright S. Evolution in Mendelian populations. Genetics. 1931;**16**:97-159

[2] Mayr E. Animal Species and Evolution. Cambridge, MA: Harvard University Press; 1963

[3] Slatkin M. Gene flow in natural populations. Annual Review of Ecology, Evolution, and Systematics. 1985;**16**:393-430

[4] Hartl DL, Clark AG. Principles of Population Genetics. 2nd ed. Sunderland, MA: Sinauer Associates; 1989

[5] Frankham R. Do island populations have less genetic variation than mainland populations? Heredity. 1997;**78**:311-327

[6] Reed DH, Frankham R. Correlation between fitness and genetic diversity. Conservation Biology. 2003;**17**:230-237

[7] Woodward G, Perkins DM, Brown LE. Climate change and freshwater ecosystems: Impacts across multiple levels of organization. Philosophical Transactions of the Royal Society B. 2010;**365**:2093-2106

[8] Black R, Bennett SRG, Thomas SM, Beddington JR. Migration as adaptation. Nature. 2011;**478**:447-449

[9] Avise JC. Molecular Markers, Natural History, and Evolution. 2nd ed. Sunderland: Sinauer Associates; 2004

[10] Allendorf FW. Genetics and the conservation of natural populations: Allozymes to genomes. Molecular Ecology. 2017;**26**:420-430

[11] Kouam EB, Ndomou M, Gouado I, Pasquet RS. Assessment of the genetic diversity of cultivated common beans (*Phaseolus vulgaris* L.) from Cameroon and Kenya using allozymes markers. Journal of Experimental Biology and Agricultural Sciences. 2017;**5**:87-97

[12] Chung MY, Chung MG. Large effective population sizes and high levels of gene flow between subpopulations of *Lilium cernuum* (Liliaceae). Biochemical Systematics and Ecology. 2014;**54**:354-361

[13] Zaghloul M, Hamrick J, Moustafa A. Allozymic and morphological differentiation between three *Ballota* species (Labiatae) growing in southern Sinai Egypt. Catrina. 2014;**9**:53-64

[14] Gormally CL, Hamrick JL, Donovan LA. Inter-island but not intra-island divergence among populations of sea oats, *Uniola paniculata* L. (Poaceae). Conservation Genetics. 2013;**14**:185

[15] Gomes T, Chora S, Pereira CG, Cardoso C, Bebianno MJ. Proteomic response of mussels *Mytilus galloprovincialis* exposed to CuO NPs and Cu^2: An exploratory biomarker discovery. Aquatic Toxicology. 2014;**155**:327-336

[16] Bradford T, Adams M, Guzik MT, Humphreys WF, Austin AD, Cooper SJB. Patterns of population genetic variation in sympatric chiltoniid amphipods within a calcrete aquifer reveal a dynamic subterranean environment. Heredity. 2013;**111**:77-85

[17] Duffy JE. Resource-associated population subdivision in a symbiotic coral-reef shrimp. Evolution. 1996;**50**:360-373

[18] Sugam K, Tridjoko T, Haryanti H, Budi SM, Folik F. Genetic variation and population structure in the humpback grouper, *Cromileptes altivelis*, throughout its range in Indonesian waters. Indonesian Fisheries Research Journal. 2017;**5**:32-38

[19] Berrebi P. Three brown trout *Salmo trutta* lineages in Corsica described

through allozyme variation. Journal of Fish Biology. 2015;**86**:60-73

[20] Kankılıç T, Tolga K, Mustafa S, Ercüment Ç. Allozyme variations in Anatolian populations and cytotypes of the blind mole rats (*Nannospalax*). Biochemical Systematics and Ecology. 2015;**59**:126-134

[21] Davis GS. Molecular systematics of thermosphaeroma isopods, and the calibration of a molecular clock [MS thesis]. Northern Arizona University; 1996

[22] Federal Register. Vol. 42, No. 251, 50 CFR part 17. 1977. pp. 65213-65214

[23] Shuster SM. Sexual selection in the Socorro isopod, *Thermosphaeroma thermophilum* (Cole and Bane). Animal Behavior. 1981;**29**:698-707

[24] Shuster SM, Miller MP, Lang BK, Zorich N, Huynh L, Keim P. The effects of controlled propagation on an endangered species: Genetic differentiation and divergence in body size among native and captive populations of the Socorro isopod (crustacea: Flabellifera). Conservation Genetics. 2005;**6**:355-368

[25] Lang BK, Kelt DA, Shuster SM. The role of controlled propagation on an endangered species: Demographic effects of habitat heterogeneity among captive and native populations of the Socorro isopod (crustacea: Flabellifera). Biodiversity and Conservation. 2006:3909-3935

[26] Axelrod DI. Age and origin of Sonoran desert vegetation. Occasional Papers of the California Academy of Sciences. 1979;**132**:1-74

[27] Shuster SM. Life history characteristics of *Thermosphaeroma thermophilum*, the Socorro isopod (crustacea: Peracarida). The Biological Bulletin. 1981;**161**:291-302

[28] Jormalainen V, Shuster SM. Microhabitat segregation and cannibalism in an endangered freshwater isopod *Thermosphaeroma thermophilum*. Oecologia. 1997;**111**:271-279

[29] Jormalainen V, Shuster SM. Female reproductive cycles and sexual conflict over precopulatory mate-guarding in *Thermosphaeroma* isopods. Ethology. 1999;**105**:233-246

[30] Jormalainen V, Shuster SM, Wildey H. Reproductive anatomy, sexual conflict and paternity in *Thermosphaeroma thermophilum*. Marine and Freshwater Behavior and Physiology. 1999;**32**:39-56

[31] Bowman TE. *Thermosphaeroma milleri* and *T. smithi*, new sphaeromatid isopod crustaceans from hot springs in Chihuahua, Mexico, with review of the genus. Journal of Crustacean Biology. 1981;**1**:105-122

[32] Sassaman C. Inbreeding and sex ratio variation in female-biased populations of a clam shrimp, *Eulimnadia texana*. Bulletin of Marine Science. 1989;**45**:425-432

[33] Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 1978;**89**:583-590

[34] Miller MA. Tools for population genetic analysis [Internet]. 1998. Available from: http://herb.bio.nau.edu/~miller/

[35] Pasteur N, Pasteur G, Bonhomme F, Catalan J, Britton-Davidian J. Practical Isozyme Genetics. Chichester: Ellis Horwood, Ltd.; 1988

[36] Christiansen B. Habitat preference among amylase genotypes in *Asellus aquaticus* (isopoda, crustacea). Hereditas. 1977;**87**:21-26

[37] Bonnel ML, Selander RK. Elephant seals: Genetic variation and near extinction. Science. 1974;**184**:908-909

[38] O'Brien SJ, Wildt DE, Goldman D, Merril CR, Bush M. The cheetah is depauperate in genetic variation. Science. 1983;**221**:459-461

[39] Bertault G, Raymond M, Berthomieu A, Callot G, Fernandez D. Trifling variation in truffles. Nature. 1998;**394**:734

[40] Sassaman C. Dynamics of a lactate dehydrogenase polymorphism in the wood louse *Porcellio scaber* Latr: Evidence for partial assortative mating and heterosis in natural populations. *Genetics*. 1978;**88**:591-609

[41] Edwards JP, Heath DJ. Dynamics of an enzyme polymorphism in the isopod, *Sphaeroma rugicauda* (leach). I. Temporal variation in genotype frequencies. Heredity. 1983;**51**:467-475

[42] Heath DJ, Riddoch BJ, Childs D, Ratford JR. Selection component analysis of the PGI polymorphism in *Sphaeroma rugicauda*. Heredity. 1988;**60**:229-235

[43] Lessios HA, Weinberg JR, Starczak VR. Temporal variation in populations of the marine isopod *Excirolana*: How stable are gene frequencies and morphology? Evolution. 1994;**48**:459-563

[44] Gentile G, Sbordoni V. Indirect methods to estimate gene flow in cave and surface populations of *Androniscus dentiger* (isopoda, Oniscoidea). Evolution. 1998;**52**:432-442

[45] Tinturier-Hamelin E. Polychromatisme et détermination génétique du sexe chez l'espéce polytypique *Idotea balthica* (pallas) (Isopode Valvifere). Cahiers de Biologie Marine. 1963;**4**:479-591

[46] Heath DJ. Seasonal changes in frequency of the "yellow" morph of the isopod, *Sphaeroma rugicauda*. Heredity. 1974;**32**:299-307

[47] Salemaa H. Geographical variability in the colour polymorphism of *Idotea baltica* (isopoda) in the northern Baltic. Hereditas. 1978;**88**:165-182

[48] Willis MJ, Heath DJ. Genetic variability and environmental variability in the estuarine isopod *Sphaeroma rugicauda*. Heredity. 1985;**55**:413-420

[49] Bowman TE. *Thermosphaeroma cavicauda* and *T. macrura*, new sphareromatid isopods from Mexican hot springs. Proceedings of Biological Society of Washington. 1985;**98**:1042-1047

Section 4

Diseases

Chapter 9

Phage Therapy for Control of Bacterial Diseases

Palaniappan Ramasamy

Abstract

Phage therapy is one of the most important control strategies envisaged for the management of bacterial diseases in the aquatic environment. There are no other effective alternative approaches for the natural control of bacterial diseases, while phage therapy remains the best method which has not yet been exploited. The occurrence, infectivity, lytic activities, therapeutic potentials, and efficacy of the bacteriophages of *Bacillus* spp./*Vibrio* spp. for control of pathogenic bacteria diseases such as *Vibrio vulnificus, V. damsela,* and *V. furnissii* in the cultures of crustaceans are presented. An ideal method for long-term storage and recovery of the lytic bacteriophages, agar bioassay method and one-step growth experiments, *in vivo* and *in vitro* experiments, and validation of the usefulness of phage therapy are described. The review highlights the occurrences of plagues of lytic phages of *Vibrio* sp. and *Bacillus* spp. and their control effects of vibriosis both *in vivo* and *in vitro* in the crustaceans, thus establishing the application and efficacy of the phages of *Vibrio*/*Bacillus* against the pathogenic *Vibrio* spp. Development of specific phage therapy or a cocktail of phages to a wide variety of systems is considered to represent an interesting emerging alternative to antibiotic therapy and vaccination.

Keywords: phage therapy, bacterial diseases, vibriosis, probiotics, bacteriophages, antimicrobials, antibiotic resistance, crustaceans, shrimp, lobster, crab, *Artemia*

1. Introduction

1.1 Global crustacean production and losses due to diseases

Global fish production was 171 million tons estimated at USD 362 billion in 2016, while aquaculture production was 80.37 million tons estimated at USD 232 billion [1–3] consisting of 54.1 million tons of finfish production, 17.1 million tons of molluscs, 7.9 million tons of crustaceans, and 938,500 tons of other aquatic animals such as turtles, sea cucumbers, sea urchins, frogs, and edible jellyfish [3]. Freshwater finfish represents half of the global aquaculture production (54%), molluscs being the second more produced aquaculture item in the world (24%) [2]. Crustaceans come next in production relevance, represented mostly by penaeid shrimps and grapsid crabs [2, 3]. Aquaculture is the world's fastest growing segment with a global increase of 5.7% per annum in shrimp production resulting in an increase of 18% by 2020, and the estimated world production of farmed shrimp is 3.5 million metric tons though the diseases, international market prices, and production costs

are the main challenges and constrains to the growth and productivity of the shrimp industry on a global level [4]. However, disease outbreaks have caused serious economic losses in several countries, and the estimated global losses due to shrimp diseases are around US$ 6 billion per annum [5, 6]. Such concerns confirm that the bacterial diseases are the most important contracting factors for development of the global aquaculture industry [7].

1.2 Bacterial diseases in crustaceans

Bacteria in the aquatic environment and the bacterial diseases, viz. vibriosis, shell diseases (chitinolytic bacteria), and gaffkemia of lobsters, are ubiquitous and are significant for the survival of crustaceans in confined habitats [8–22]. Diseases,

Diseases	Hosts	Causative bacterial species
Vibriosis	*Penaeus monodon*, *P. merguiensis*, and *P. indicus* (eggs, larvae, postlarvae, juveniles, and adults); *Litopenaeus vannamei*, *Macrobrachium*, lobster *Homarus americanus*, crab *Portunus trituberculatus*	*Vibrio harveyi*, *V. splendidus*; *Vibrio harveyi*, *V. alginolyticus*, *V. parahaemolyticus*, *V. anguillarum*, *V. furnissii*, *V. mimicus*, *V. damsela* [7, 13–22]
Bacterial fouling of surfaces with filamentous bacterial disease	*Penaeus monodon*, *P. merguiensis*, *P. indicus*	*Leucothrix* sp., *Thiothrix* sp., *Flexibacter* sp., *Cytophaga* sp., *Flavobacterium* sp. [7]
Shell disease, brown/black spot, black gill, black rot/ erosion, blisters, necrosis of appendages	Crabs and shrimp, white and brown shrimp *Penaeus monodon*, *P. merguiensis*, *P. indicus*	Chitonoclastic bacteria *Vibrio* spp. infections *Vibrio*, *Aeromonas*, *Pseudomonas*, *Flavobacterium* [7, 8, 11]
Chitinolytic bacterial disease, shell disease, box burnt disease, bacterial shell disease	*Cancer* spp., *Callinectes sapidus*, other crabs; lobsters, shrimps, and crayfish	Chitinolytic or chitinoclastic bacteria (Gram-negative), viz. *Vibrio* spp., *Pseudomonas* spp., and *Aeromonas* spp. [7, 9–11, 13, 17, 20–22]
Milky hemolymph disease (milky hemolymph syndrome [MHS])	Spiny lobster *Panulirus* spp., *Panulirus ornatus*, *P. homarus*, and *P. stimpsoni*; *Litopenaeus vannamei* (*Penaeus monodon, Carcinus maenas*)	Rickettsia-like bacterium, a-*Proteobacteria*, *Streptococcus* sp., [7, 10–12]
Gaffkemia, septicemia	Lobsters *Homarus americanus*	*Gaffkya homari* [7, 9–11]
Bacteremias	Bacterial diseases of crabs	*Vibrio*, *Aeromonas*, *Rhodobacteriales*-like organism, *Vibrio cholerae*, *Vibrio vulnificus*, chitinoclastic bacteria, *Rickettsia* intracellular organisms, chlamydia-like organism, *Spiroplasma*, chitinoclastic bacteria, *Rickettsia* intracellular organisms, chlamydia-like organism, and *Spiroplasma* [7]
Fungal diseases	*Penaeus monodon*, [larval (nauplii, zoea, and mysis) Indian tiger prawn] *Macrobrachium rosenbergii*	*Lagenidium callinectes* [16, 17]

Table 1.
Bacterial diseases, causative organisms, and their crustacean hosts.

causative organisms, and their crustacean hosts are listed in **Table 1**. Gaffkemia of lobsters is caused by *Aerococcus viridans* var. *homari* and is the root cause of mass mortalities of lobsters, while crabs, viz. *Cancer borealis* and *C. irroratus*, serve as reservoir hosts of *Aerococcus viridans* [8–13]. *Vibriosis* causes mass mortalities in several crustaceans such as penaeid shrimp *Penaeus monodon* and *P. japonicus*, fresh water prawn *Macrobrachium*, lobster *Homarus americanus*, blue crabs *Callinectes sapidus*, rock crabs *Cancer irroratus*, and shore crab *Carcinus maenas*. Shell diseases are caused by chitinolytic bacteria which were encountered in English prawn *Palaemon serratus*; American lobsters *Homarus americanus*; penaeid shrimp; king crabs, *Paralithodes camtschaticus* and *Paralithodes platypus*; and tanner crabs *Chionoecetes tanneri*, and these crustaceans are affected by rust diseases which are caused by chitin-destroying bacteria [8–22]. Significant mortalities of larval, post-larval, and adult crustaceans, viz. shrimp *Penaeus monodon*, *Litopenaeus vannamei*, and *Macrobrachium*, lobster *Homarus americanus*, and crab *Portunus trituberculatus*, are caused by common pathogens such as *Vibrio harveyi*, *V. alginolyticus*, *V. parahaemolyticus*, *V. anguillarum*, *V. furnissii*, *V. mimicus*, *V. damsela*, *Pseudomonas*, and *Aeromonas* [9–22]. Infectious diseases caused by *Vibrio* species represent the greatest challenges that cause vibriosis with considerable economic losses, and that is the most overwhelming problem in aquaculture, shrimp, and crustaceans [7–101].

2. Phage therapy

Phage therapy is a prospective ideal therapy for vibriosis in aquaculture of crustaceans. Bacteriophages are defined as bacterial viruses that can infect cells, multiply in, cytolyse, and destroy susceptible bacteria. Bacteriophages are viruses of bacteria which have a natural ability to target, infect, and destroy their host cells of a particular bacterial species or groups or even unrelated bacteria, and thus they play a major role in controlling their target bacterial population density in nature [23–60]. They are both omnipresent and copious in the aquaculture environment, especially in seawater, in which the total numbers of viruses normally surpass the bacterial cell concentration by a factor of 10 [25]. "Most phages reproduce upon entering a cell and in that process kill their host. They encode two families of proteins, holins, and lysins, which allow the phage progeny to burst through the bacterial cell wall and go off in search of new hosts. But the cell is already dead by the time that happens in terms of lethality; the lysis is just a matter of burning down the house for good measure." An estimated 10^8 strains of phage with approximately 10^{31}–10^{32} phages are known to occur in the biosphere at any given time [23]. Bacteriophages are used for the isolation and identification of specific bacteria to help in the diagnosis of the bacterial diseases, to kill antibiotic-resistant, virulent bacteria through a natural phenomenon called lysogeny, whereby one of the phage-infected bacteria in a colony kills another uninfected bacterium through phage missiles or antibacterial peptides [24, 25, 36–39, 41].

2.1 Phage therapy, an alternative to antibiotics

Due to their specific antibacterial activities and significance of the phage therapy as an alternative to antibiotics, bacteriophage therapy is re-emerging, and consequently this has become a potentially novel and useful concept to kill even intracellular pathogenic bacteria and warrant future development. Bacteriophage therapy has been extended from medical applications into the fields of agriculture, aquaculture, and the food industry [28–30, 39]. Bacteriophages specific for *Vibrio* spp. have been described [36–41]. Bacteriophages are known to infect >140 bacterial

genera, and they are the most valuable and ubiquitous (10^{31}) phage organisms in the world [26–35, 40–45]. Earliest description of phages and their antibacterial activity has been independently demonstrated [62]. D'Herelle [33] published a comprehensive account of phages, and hence the International Bacteriophage Institute was established in Tbilisi, Georgia, in 1923, now called as "the George Eliava Institute of Bacteriophages, Microbiology and Virology" [32, 34], which is still involved in researching phage therapy applications and supplies phage for the treatment of various bacterial infections. D'Herelle's first phage therapy experiments against bacterial dysentery were exceptionally successful and were very promising in the removal of the infectious organisms [32, 34]. However some of the results of the early phage therapy experiments of infectious host organisms were known to be contradictory with reports of both success and failure. Whenever failures occurred in phage therapy, they were attributed to a range of factors. They include unsatisfactory understanding of phage biology, inadequate experimental technical knowledge, poor quality of phage preparations, and a lack of understanding of the causes of illness being treated. The discovery of such specific bacteriophages were at first considered to become powerful beneficial therapeutic agents against pathogenic microorganisms but could not be put into practice because of the dawn of antibiotic era experiencing overuse/misuse of antibacterial medication that resulted in the development of untreatable antibiotic resistance [32, 36]. Commercialization of antibiotics in the Western countries in the 1940s had led to a simultaneous decline in the use of phages as human therapeutics, while in some of the Eastern European countries, exploitation of phage therapy was continued either alone or in combination with antibiotics [19, 34, 61–73]. The experimental phage therapy could be an alternative to antibiotics and replace them when they fail for the treatment of chronic infections, and such a successful eradication of drug-resistant bacteria was documented [19, 42, 61–73]. Moreover, the significantly decline costs of phage therapy constitute an important additional battle for its wider application in the current era of a worldwide circumstance in antibiotic resistance. The efficacy of phage therapy is well recognized and demonstrated in a few cases [32, 64–66]. Even a single dose of phage was reported to be much more effective than multiple doses of antibiotics such as ampicillin, tetracycline, and chloramphenicol [66]. The use of phage therapy to control fish pathogens has also been reported [23, 24, 42, 43]. Phage lysins have been used as potential therapeutics for treatment of bacterial infections [44]. Furthermore, the US Food and Drug Administration has approved commercial phage preparations to prevent bacterial contaminations. Such developments have prompted to explore the possibilities of using bacteriophages to control bacterial infections in crustacean aquaculture [36–41]. Further cautious phage collection and perfect experimental conditions for phage propagation and purification, route and timing of phage administration, and environmental monitoring of phage for use are needed. Such a focus may help in further development of probiotics, phage therapy applicable to a wide variety of systems, which is considered to signify an emerging alternative to antibiotic therapy and vaccination.

2.2 Probiotics in crustacean cultures

Probiotics are defined as applications of whole or components of microorganisms or "a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment" [74–93]. A probiotic is a live microbial feed supplement which benefits the host

animal by improving its intestinal microbial balance [82]. Lactic acid bacterium *Bacillus* S11 was tested as probiotics and has been proven as antagonists of shrimp pathogens [75]. *Bacillus* spores were used as biocontrol agents to reduce *Vibrio* species in shrimp culture facilities [83]. The inhibitory activity of *Bacillus subtilis* BT23, isolated from shrimp culture ponds, is against pathogenic *Vibrio harveyi* under *in vitro* and *in vivo* conditions [39, 73]. The concept of probiotics is exploited to "augment" naturally occurring pathogenic bacterial population to increase the growth rate and control diseases of organisms in aquaculture such as fish/shellfish [84, 85]. Several microorganisms have been evaluated as probiotics which have been shown to be successful in the larval stages of the aquatic organisms in preventing the diseases, improving digestion and growth [82, 84, 87]. Some of the proposed mechanisms that provide protection against pathogens involve the production of inhibitory compounds, competition for essential nutrients and adhesion sites, enhancement of disease resistance, and modulation of host immune responses [82, 87]. A probiotic containing a combination of several different bacteria has been shown to be more efficient at controlling bacterial pathogens [82, 90, 91]. Probiotics are known to act and inhibit the growth and proliferation of bacterial pathogens both *in vivo* and *in vitro* by generating antibacterial compounds such as bacteriocin, siderophores, lysozymes, proteases, hydrogen peroxide, antibiotics, and organic acids [91]. In aquaculture, some microorganisms are more beneficial to host organisms in reducing the incidence of diseases though the factors and mechanisms which mediate the benefits to the host are poorly understood. Future studies should be focused on evaluating the mechanisms by which the probiotics interact with the host and pathogens and their biology.

2.3 Proprobiotics

Proprobiotic is defined as a synbiotic comprising pre- and probiotics involving a combination of strains of nonpathogenic bacteria and yeast so as to provide supplementary nutrients and to protect against invading pathogens. Commercially available proprobiotics are known to competitively exclude the potential pathogen through the use of proprobiotics in aquaculture. Effects of Ergosan and Vibromax are used to prevent vibriosis in *Litopenaeus vannamei* [42, 43]. Probiotics (e.g., Arda-Tek, Australia, viz. DMS1000, S-1100, DMS-2001, DMS-2002, DMS-2004, etc., Wunapuo-15 of Team Aqua Corporation) are used to reduce the number of Gram-negative pathogenic bacteria including *Vibrio* in the water and to maintain stable water quality, to stabilize plankton, to reduce rate of sludge buildup on the pond bottom, and to digest microbial slime [73, 82, 84–91]. To assess the actual impact of these probiotic products in the field, it is fundamental to determine the efficacy of the probiotics and the continuance of their application. The potency of the probiotic products against selected pathogenic bacteria (and viruses) also needs to be critically evaluated. The application of some beneficial bacteria has been an option in aquaculture to achieve a number of benefits, viz. to reduce mortality in shrimp, to enhance production and increase harvest yield and eliminate antibiotic use, and to enhance immunity and disease resistance in black tiger shrimp *Penaeus monodon* by a probiont bacterium (*Bacillus* S11) [84–91]. Immunity enhancement occurs in black tiger shrimp *Penaeus monodon* by a probiont bacterium (*Bacillus* S11), and the immune stimulator capacity of probiotics may be affected by factors such as source, type, dose, and duration of supplementation [80, 85]. The use of probiotic mixtures consisting of *Bacillus tequilensis* and *B. endophyticus* along with commercial probiotics was shown to be beneficial in altering the bacterial community of larval shrimp *Litopenaeus vannamei* when the hosts were challenged with *Vibrio parahaemolyticus*. Similarly application of *Bacillus* sp. promoted cellular and

humoral immune resistances and provided disease protection in tiger shrimp *P. monodon*. The effects of probiotics such as *Bacillus cereus*, *Paenibacillus polymyxa*, and *Pseudomonas* sp. PS-102 as biocontrol agents against pathogens of various *Vibrio* species in shrimp were evaluated. Growth promoter probiotics have been used in aquaculture to enhance the growth of cultivated species, whereas their side effects if any on the host need to be investigated. The probiotics was shown to increase the survival and growth of white shrimp (*Litopenaeus vannamei*), and the production increased by 35%, whereas with the use of antimicrobials, it decreased by 94% and thus demonstrated the significance of endemic *Bacillus* probiotics on the improvement of the health of larval shrimp [71, 73]. The efficacy of the non-hemolytic probiotic *Bacillus* strains against pathogenic *Vibrio* species, viz. *Vibrio campbellii*, *V. vulnificus*, *V. parahaemolyticus*, and *V. alginolyticus* on the growth of the larval shrimp was tested by using a daily concentration of 1×10^5 cfu ml^{-1} with initial bioassay density of 225 nauplii L^{-1}, and the treatments promoted a considerable increase in survival and growth of the larval shrimp compared to the control, and thus the study has established a significant antagonistic activity of the probiotic *Bacillus* strains against the *Vibrio* species [48]. Various strains of *Bacillus*, or a commercial product made from *Bacillus* sp., *Saccharomyces cerevisiae*, *Nitrosomonas* sp., and *Nitrobacter* sp., used as probiotics show prevention of infection and promotion of growth rate of all the stages of the white shrimp *Litopenaeus vannamei* Boone and *Fenneropenaeus indicus* when the diet was supplemented with 50 g of probiotic kg^{-1} of food. The use of probiotic *Shewanella* algae in shrimp farms is found to be safe for the consumer of shrimp [92]. A scientific rational approach for the evaluation of aquaculture probiotics and guidelines is needed for their use and safety.

2.4 *Bacillus* for the control of vibriosis

Pathogenic *Vibrio* spp. were controlled by cell-free extracts of *Bacillus* under *in vitro* and *in vivo* conditions indicating that probiotic treatment offers a promising alternative to the use of antibiotics in shrimp aquaculture [41, 63, 70, 85]. Though probiotic bacteria are currently used to improve the health of the shrimp ponds and increase productivity and reduce mortality of prawns, little attention has been paid to bacteriophages preying on the probiotic bacteria of crustacean culture such as *Vibrio*/*Bacillus* spp. from aquaculture systems. A special focus needs to be given to bacteriophages infecting probiotic bacteria to explore the antibacterial potential of bacteriophages of *Bacillus* spp., *Vibrio* spp., and other bacteria. The existence of bacteriophages in the probiotic *Bacillus* sp. and *Vibrio* spp. controlling the infectivity of the *Vibrio* spp. with a significant reduction in the mortality of infected shrimp was demonstrated [37, 41]. Anti-*Vibrio* activities of *Bacillus* spp. mainly due to the production of bacteriocin or bacteriocin-like substances have been reported from *B. subtilis*, *B. megaterium*, *B. stearothermophilus*, *B. licheniformis*, *B. thuringiensis*, *B. thermovorans*, and *B. cereus* [16, 22, 45, 47, 68, 69, 83]. *P. monodon* larvae fed with *Bacillus* S11 showed 100% survival after challenge with pathogenic *V. harveyi*, whereas only 26% control animals survived.

2.5 Antimicrobials

Vibrio species are Gram-negative curved rod-shaped bacteria that belong to the *Vibrionaceae* family, and they naturally inhabit the estuarine, coastal, and marine environment worldwide. *Vibrio* species occur as the dominant flora in all developmental stages of *Penaeus monodon*, and they have been described as the causal pathogens. *Vibriosis* is a severe bacterial disease in penaeid shrimp and is responsible

for large-scale losses in the aquaculture industry, leading to prophylactic as well as therapeutic use of antimicrobials [18–22, 70–73]. Antibiotics are used in shrimp farming to control or treat bacterial disease outbreaks with an expected 100,000–200,000 tons of antibiotics being consumed in the world every year [57]. Oxytetracycline, tetracycline, quinolones, sulfonamides, and trimethoprim are among the antimicrobials utilized to control bacterial infections in the aquaculture industry. Indiscriminate prophylactic uses of diverse antibiotics in the shrimp hatcheries/farms have resulted in antibiotic resistance of the bacteria causing mass mortalities of the hosts [73–75]. The potential negative consequences are the development of drug-resistant bacteria and reduced efficacy of antibiotic treatment. Shrimp farmers are exceedingly dependent on various antibiotics as a preventive measure against shrimp bacterial infections with 14% of farmers using antibiotics on a daily basis in their farms [22].

2.6 Antibiotic-resistant bacterial infections in crustaceans

All of the 121 isolates of *Vibrio* spp. were found to be 100% resistant to ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin, penicillin G, and furazolidone and partially resistant to cefaclor, streptomycin, rifampicin, oxytetracycline, nalidixic acid, cefotaxime, and chlorotetracycline [14, 16]. Molitoris et al. [14] reported a high degree of resistance to ampicillin and furazolidone, and incidence of resistance to chloramphenicol, neomycin, and gentamycin has also been detected [16, 18–20, 22, 46, 69]. Multidrug-resistant *Vibrio harveyi* isolated from black gill-diseased *Fenneropenaeus indicus* and antibacterial activity against pathogenic *Vibrio harveyi* and its protective efficacy on juvenile *F. indicus* were reported. In vitro susceptibility of antibiotics against *Vibrio* spp. and *Aeromonas* spp. isolated from *Penaeus monodon* hatcheries and ponds and the effect of probiotics, antibiotics, and pathogenicity of *Listonella anguillarum*-like bacteria isolated from *Penaeus monodon* culture systems and antibiotic-resistant *Vibrio* spp. were commonly isolated from hatchery-reared postlarvae compared to farm-reared *P. monodon* [61, 62, 72, 73, 79, 85]. The unrelenting use of antibiotics against diseases in human beings and other life forms may pollute the aquatic system, and their impact on developing antibiotic-resistant *Vibrio* sp. may be a serious threat in addition to the use of antibiotics in aquaculture farms [63, 70]. Control of the bacterial diseases depends on improvement of management practices to minimize the risk of introduction of infectious agents into aquaculture systems and to reduce predisposing factors such as overcrowding and overfeeding.

2.7 Antibiotic-resistant genes (ARGs)

A study assessed a variety of antibiotic-resistant bacteria and detected the presence of their resistance genes from mariculture environments. A variety of antibiotics that were used in aquaculture have led to the occurrence of antibiotic-resistant genes in bacteria, and in these bacteria many different ARGs can be found, and they are β-lactam- and penicillin-resistant genes *penA* and *blaTEM-1*; chloramphenicol-resistant genes *cat*I, *cat*II, *cat*III, *cat*IV, and *floR*; tetracycline-resistant genes *tatA*, *tatB*, *tatC*, *tatD*, *tatE*, *tatG*, *tatH*, *tatJ*, *tatY*, and *tatZ*; and many more [65, 68]. ARGs can be transferred among bacteria via conjugation, transduction, or transformation. The widespread emergence of antimicrobial resistant bacteria worldwide has become a major therapeutic challenge. Therefore there is a need for development of novel non-antibiotic approach such as phage therapy biocontrol agents to fight against resistant bacterial infections due to the shortage of new antibiotics in developmental pipeline [19, 61–73]. Thiel [25] stated that phage therapy is "a nearly

forgotten therapy that may yet re-emerge as a savior to this accelerating crisis of antibiotic resistance. For every bacterium known on this planet, there are legions of bacteriophages—tiny viruses that seek out bacteria and use them as a breeding ground, almost invariably destroying their prokaryotic host in the process. That makes them harmless to even to nontarget bacteria—distinguishing them from broadspectrum antibiotics, which, when they work, can wipe out beneficial flora along with a troublesome infection." The phage specificity has its drawbacks as there is a requirement of the right match between phage and bacteria which needs to be determined for the phage therapy to work. There has been renewed concern in the application of bacteriophage as a non-antibiotic approach to control bacterial infections in various fields including human infections, food safety, agriculture, and veterinary applications. However the guideline data on the use of phage therapy applied to invertebrates, like shrimp and other crustaceans, are nonexistent [64, 68, 92, 93].

2.8 Bacteriophage therapy for biocontrol of vibriosis in crustaceans

A bacteriophage isolated from a shrimp hatchery was shown to infect *V. harveyi*, signifying its potential as a biocontrol agent of luminous vibriosis. In vitro treatments of bacteriophages were shown to exhibit a significant reduction (2–3 log units) in the number of *V. harveyi* host cells [41, 46, 48, 49]. These studies showed that bacteriophages could be used for biocontrol of *V. harveyi* and that bacteriophage therapy could be effective as an alternative to antibiotics in the control of luminous vibriosis in shrimp hatchery systems [23–69]. In vitro experiments confirmed that bacteriophages could be effectively used *in vivo* as biological agents to control *Vibrio* sp. in aquaculture systems [36–39, 41, 46, 48, 49]. Investigation on the occurrences of luminescent *V. harveyi* and their bacteriophages in shrimp showed that the presence of low concentrations of bacteriophages in the larval rearing tank waters could not prevent the development of luminous vibriosis [46–49], and this study showed that the presence of optimal concentration of phages is required for effective reduction of the pathogenic bacteria. A lytic spectrum of bacteriophages (Viha1, Viha2, Viha3, Viha4, Viha6, Viha7) occurring in nature exhibited a wide spectrum of activity against *V. harveyi*, suggesting their potential as agents for biocontrol of vibriosis in aquaculture environments [51]. A lytic phage (PW2) was isolated and characterized under controlled conditions in the laboratory from the host bacterium *V. harveyi* CS101 [49], and the useful bacteriophages for the biocontrol of vibriosis have been explored. A probiotic strain *V. alginolyticus* reduced the diseases and mortality of infected *P. monodon* with *A. salmonicida*, *V. anguillarum*, and *V. ordalli* [49]. A soil bacterial strain, PM-4, was shown to exhibit *in vitro* inhibitory effect against *V. anguillarum* and to promote the growth of *P. monodon* nauplius [53]. Inoculation of *Bacillus* S11, a saprophytic strain, resulted in greater survival of the post-larval *P. monodon* that were challenged with pathogenic luminescent bacterial culture [51]. These works strongly suggest an effective control of microflora in crustaceans can be achieved in aquaculture environments by bacteriophage-producing bacteria. The crustacean host, causative agent, diseases, and source of bacteriophages/bacteria are listed in **Table 2**.

2.9 In vitro and *in vivo* effects of *Bacillus* against vibriosis in shrimp

In vitro and *in vivo* antagonistic effects of *Bacillus* against the pathogenic *Vibrio* species were evaluated [40]. Cell-free extracts of *Bacillus subtilis* BT23 were shown to exhibit inhibitory effects against the growth and proliferation of *Vibrio harveyi* isolated from black gill-diseased *Penaeus monodon*. The probiotic effect of *Bacillus*

Crustacean host	Bacterial agent	Infection/ disease	Bacteriophage	Source of the bacteriophage	Efficacy of bacteriophage	Outcome of the phage therapy	References
Shrimp larvae *Penaeus monodon*	*Vibrio harveyi*	Luminous vibriosis	*Myoviridae* (VHLM)	Extracted from a toxin-producing strain of *V. harveyi* isolated from moribund prawn	Vibriolysis	VHML showed a narrow host range with a preference for *V. harveyi* rather than 63 other *Vibrio* isolates and 10 other genera	[100]
Shrimp larvae *Penaeus monodon*	*Vibrio harveyi*	Luminous vibriosis	*Siphoviridae*	Shrimp farm waters from the West coast of India	18-day-old PL shrimp were challenged with the bacteria (10^5 cells ml^{-1}, laboratory trial: (1) bacteriophage suspension (10^9 pfu ml^{-1}) was added initially; after 24 h (another 0.1 ml), (2) only once initially with 0.1 ml of the phage suspension; (3) no addition. Hatchery trial (1) treatment with bacteriophage (10^9 pfu ml^{-1}) at the rate of 200 ppm daily so that phage concentration in the water was 2^9–10^5 pfu ml^{-1}; (2) treatment with antibiotics (oxytetracycline 5 ppm, kanamycin 10 ppm daily); (3) no treatment	Enhanced survival (80%) of *P. monodon* larvae on treatment with two doses of bacteriophage when compared with the control (25%). Hatchery trial: survival in the control tank was only 17%, while in antibiotic-treated tanks, survival was 40%; in the bacteriophage-treated tank, survival was 86%. Bacteriophage therapy has an excellent potential in management of luminous vibriosis in aquaculture systems	[48]
Larval shrimp *Penaeus monodon*	*Vibrio harveyi*	Luminous vibriosis	*Siphoviridae*	Three from oyster tissue and one from shrimp hatchery water	Hatchery tanks, with post-larval five-stage larvae, presenting luminescence and mortality, were used. Bacteriophage treatment (two tanks): one suspension (2^9 10^6 pfu ml^{-1}) was added by day following the order: Viha10, Viha8, Viha10, and Viha8 chemotherapy (two	Bacteriophage treatment resulted in over 85% survival of *Penaeus monodon* larvae. The normal hatchery practice of antibiotic treatment resulted in a survival range from 65 to 68%. This study shows that bacteriophages could be used for biocontrol of *V. harveyi*	[46]

Crustacean host	Bacterial agent	Infection/ disease	Bacteriophage	Source of the bacteriophage	Efficacy of bacteriophage	Outcome of the phage therapy	References
					tanks): oxytetracycline (5 mg L^{-1}), kanamycin (10 mg L^{-1})		
Penaeid shrimp *P. monodon*	*Vibrio harveyi*	Luminous vibriosis	Seven bacteriophages specific to *Vibrio harveyi* (Viha1 to Viha7), six from *Siphoviridae* and one *Myoviridae* (Viha4)	Coastal aquaculture systems like shrimp farms, hatcheries, and tidal creeks along the East and West coast of India		All the phages were found to be highly lytic for *V. harveyi*. The phages exhibited a different lytic spectrum for a large number of bacterial isolates tested. Three of the phages (Viha1, Viha3, and Viha7) caused 65% of the strains to lyse, while Viha2, Viha4, and Viha6 caused 40% of the host strains to lyse. Only Viha5 had a narrow spectrum (14%). Six of the seven phages isolated had a broad lytic spectrum and could be potential candidates for biocontrol of *V. harveyi* in aquaculture	[51]
Shrimp *P. monodon*	*Vibrio harveyi*	Luminous vibriosis	*Siphoviridae* (VH1 to VH8)	Shrimp farm	*In vitro* experiment	All the isolates of bacteriophage (VH1–VH8) caused lysis of the host bacterial cells within 2 h. The propagation curve for each phage showed a burst time from 1 to 10 h. Bacteriophages of *Vibrio* sp. shall be effectively used *in vivo* as biological agents to control these pathogenic bacteria in aquaculture systems	[39]
Shrimp *P. monodon*	*Vibrio harveyi* CS101	Luminous vibriosis	*Siphoviridae* (phage PW2)	Shrimp pond water		Phage adsorption rate increased rapidly in the first 15 min of	[52]

Crustacean host	Bacterial agent	Infection/ disease	Bacteriophage	Source of the bacteriophage	Efficacy of bacteriophage	Outcome of the phage therapy	References
						infection to 80% and continued to increase to 90% within 30 min of infection. The stability of phage PW2 was dependent on temperature and pH. It was inactivated by heating at 90°C for 30 min and by treating at pH 2, 3, 11, and 12. One-step growth curve and latent and burst periods were 30 and 120 min, respectively, with a burst size of 78 pfu per infected center. Six structural proteins were detected	
Larval shrimp *P. monodon*	*V. harveyi*	Luminous vibriosis	ϕH17-5c, ϕH17-7b, ϕH17-8b, and ϕH17-9b	Secluded from shrimp farm water from the West coast of India and demonstrated to exhibit a broad lytic activity against *V. harveyi* isolates	In a set of laboratory experiments, post-larval *Penaeus monodon* was exposed to 10^6 cfu ml^{-1} cells of *Vibrio harveyi* and was treated with 100 ppm phage which has led to a drastic reduction of *Vibrio harveyi* counts with 86% survival of the infected larvae, while the survival of the phage-untreated larvae was 25%	In the antibiotic (oxytetracycline 5 ppm, kanamycin 100 ppm/ day)-treated hatchery tanks, an initial reduction of luminous bacterial counts were shown, and after 48 h the bacterial count increased to 106 ml^{-1} showing the luminous vibriosis and mortality of the nauplii of *Penaeus monodon* with 40% larval survival. In contrast, in the bacteriophage-treated tank, the larval survival was 86%, and the survival rate in the control tank was only 17%	[48, 49]
Shrimp, *P. monodon*	*V. vulnificus*	Vibriosis	Phages VV1, VV2, VV3, and VV4 from *V. vulnificus*	VV1, VV2, VV3, and VV4 phages were detected from	In vitro experiments show successful potential phage therapy; lytic *V. vulnificus* phages	*V. vulnificus* phages exhibited a broad lytic spectrum and potential biocontrol of luminous	[36–39]

Crustacean host	Bacterial agent	Infection/ disease	Bacteriophage	Source of the bacteriophage	Efficacy of bacteriophage	Outcome of the phage therapy	References
				shrimp aquaculture system	infect a wide variety of other *Vibrio* spp./isolates	vibriosis in the shrimp aquaculture system	
Phyllosoma larvae of the tropical rock lobster *Panulirus ornatus*	*V. harveyi*	Luminous vibriosis	Six bacteriophages from *Siphoviridae* (VhCCS-01, VhCCS-02, VhCCS-04, VhCCS-06, VhCCS-17, and VhCCS-20) and two from *Myoviridae* (VhCCS-19 and VhCCS-21)	Isolated from an epizootic in aquaculture-reared larval phyllosomas of the ornate spiny lobster *Panulirus ornatus* water samples from discharge channels and grow-out ponds of a prawn farm	Exhibited a clear lytic activity against *V. harveyi* (1) Addition of phage VhCCS-06 (1 ml) 2 h after inoculation; (2) addition of phage VhCCS-06 (1 ml) 6 h after Bacteria-free supernatants were obtained by centrifugation at 10,000 *g* for 15 min and by filtration of the aliquots of the enriched water samples; supernatants (10 ll) were inoculated onto NAMS plates, grown at 28°C for 24 h, and the bacteria-free supernatants were stored at 4°C	Exhibited a clear lytic activity against *V. harveyi* with no apparent transducing properties. Phages of VhCCS-19 and VhCCS-21 are ***Myoviridae*** bacteriophages and lysogenic and induce **bacteriocin production** in the host bacteria (*V. harveyi* strain 12); ***Siphoviridae*** phage (VhCCS-06) delayed the entry of a broth culture of *V. harveyi* strain 12 into exponential growth, though it could not prevent the overall growth of the bacterial strain. This effect was due to the multiplication of **phage-resistant cells** of *V. harveyi*. Phage resistance is an obstacle to the use of phage as therapeutic agents. The isolated phages exhibited lytic activity against strains of *V. harveyi*, a primary pathogen of phyllosoma of the tropical rock lobster, *P. ornatus*. These phages can be used as a biocontrol agent to combat vibriosis in the rearing	[55]

Crustacean host	Bacterial agent	Infection/ disease	Bacteriophage	Source of the bacteriophage	Efficacy of bacteriophage	Outcome of the phage therapy	References
						system of phyllosoma of the tropical rock lobster, *P. ornatus*	
Live prey *Artemia salina*	*V. alginolyticus* strain V1	Vibriosis	Two novel bacteriophages *φ*St2 and *φ*Grn1	*Vibrio alginolyticus* strain V1, isolated during a vibriosis outbreak in cultured seabream	In vitro cell lysis experiments against the bacterial host *V. alginolyticus* strain V1 and also against 12 *Vibrio* strains originating from live prey *Artemia salina* cultures, viz. *V. anguillarum*, *V. harveyi*, *V. alginolyticus*, *V. ordalii*, *V. parahaemolyticus*, *V. splendidus*, and *V. owensii).* It indicated a strong lytic efficacy of the 2 phages	In vivo administration of the phage cocktail consisting of *φ*St2 and *φ*Grn1, directly on live prey *A. salina* cultures, has led to a 93% decrease of *Vibrio* population, viz. *V. anguillarum*, *V. harveyi*, *V. alginolyticus*, *V. ordalii*, *V. parahaemolyticus*, *V. splendidus*, and *V. owensii*, after 4 h of treatment	[98, 99]
Brine shrimp nauplii *Artemia franciscana*	*V. parahaemolyticus*	Vibriosis of *Artemia franciscana*	VPMS1 phage	VPMS1 is a lytic phage of *Vibrio parahaemolyticus*, isolated from a marine clam	*V. parahaemolyticus*-infected brine shrimp nauplii were treated with a single dosage of VPMS1 phage, which was effective enough to eliminate the adverse effects of *V. parahaemolyticus* in brine shrimp. Efficacy was not affected by the reduction in the dosage	The phage therapy was successful in preventing vibriosis; a single dosage of VPMS1 phage was effective enough to get rid of the adverse effects of *V. parahaemolyticus* in brine shrimp; the beneficial effects of the therapy were compromised if the application of phages was delayed	[97]
Penaeus monodon rearing waters in shrimp ponds in Palk Strait, South East coast of India	*Vibrio parahaemolyticus*	*Vibrio parahaemolyticus* and its potential lytic phage from *Penaeus monodon*	Lytic phage (VVP1) belongs to the *Myoviridae* family	Lytic phage (VVP1) able to infect strains of N1A and N7A, *V. parahaemolyticus*, and strains of N3B and N13B *Vibrio alginolyticus*	One-step growth experiments, multiplication and host range, and pH and temperature stability of the lytic phage (VVP1) were shown; the phage showed protective biocontrol effects in reducing the pathogenic *V.*	*P. monodon* larvae infected with *V. parahaemolyticus* showed enhanced survival of the larvae in the presence of phage treatment at lytic phage (VVP1), 2.3×10^{10} PFU ml^{-1}, when compared with the control and	[101]

Crustacean host	Bacterial agent	Infection/ disease	Bacteriophage	Source of the bacteriophage	Efficacy of bacteriophage	Outcome of the phage therapy	References
					parahaemolyticus in infected shrimp larvae	showed that the application of phage therapy is a useful strategy to prevent and eliminate or reduce shrimp pathogenic *V. parahaemolyticus* in the aquaculture system	
V. alginolyticus phages were isolated from seawater samples after enrichment	*V. alginolyticus*, *Vibrio alginolyticus*, a zoonotic pathogen that causes mass mortality in aquatic animals and infects humans	Vibriosis, a zoonotic pathogen causing mass mortality in aquatic animals and infecting humans	Phage pVa-21, *Myoviridae*	Phage pVa-21 infects bacteria belonging to the family *Vibrionaceae*, *viz. V. alginolyticus* and *V. harveyi*, and could not infect *V. parahaemolyticus*, *V. anguillarum*, *V. campbellii*, and *V. vulnificus*	Bacteriophage pVa-21 belongs to *Myoviridae*, characterized as a candidate biocontrol agent against *V. alginolyticus*. It exhibits planktonic or biofilm lytic activity and showed stability under various conditions. It has latent period and burst size approximately 70 min and 58 plaque-forming units/cell, respectively. Phage pVa-21 can inhibit bacterial growth in both the planktonic and biofilm states. The phage is related to the giant phiKZ-like phages, classified as a new member of the phiKZ-like bacteriophages that infect bacteria belonging to the family *Vibrionaceae*	Infect bacteria, viz. *V. alginolyticus* and *V. harveyi* in both the planktonic and biofilm states	[101]

Table 2.
Crustacean host, bacteria, disease, and source of bacteriophages/bacteria for bacteriotherapy.

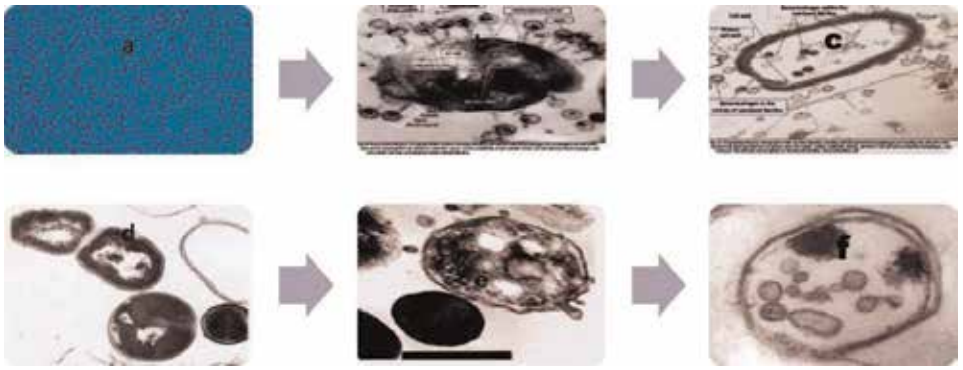

Figure 1.
(a) Light micrograph of uninfected Bacillus sp.; (b) TEM micrograph showing phages on the surface of the infected Bacillus. (c) TEM micrograph of phage-lysed bacterial cell. (d) TEM micrograph of uninfected Vibrio cells. (e) TEM micrograph of phage-infected Vibrio cell. (f) TEM micrograph of phage-lysed Vibrio cell.

was tested by exposing shrimp to *B. subtilis* BT23 at a density of $10^6/10^8$ cfu ml^{-1} for 6 days before a challenge with *V. harveyi* at $10^3/10^4$ cfu ml^{-1} for 1 h infection (**Figure 1a-f**). Probiotic treatment of *B. subtilis* BT23 showed a 90% reduction in accumulated mortality of *P. monodon*. Pathogenic *Vibrio* species were controlled by *Bacillus* under *in vitro* and *in vivo* conditions, and the results indicated that probiotic treatment offers a promising alternative to the use of antibiotics in shrimp aquaculture.

The occurrence of phages in infected cells of *Bacillus* sp. is illustrated (**Figure 1a-c**). Each bacteriophage particle of the bacterium *Bacillus* sp. presented as electron-dense objects comprising a distinctive head with a flexible noncontractile tail. The preparations consisted of 30 min–2 h exposures of *Bacillus* cells to *in vitro* phage infection, and the TEM of such experimentally phage-infected cells showed actively adsorbing and infecting phages onto the surface of bacterial cell membrane (**Figure 1b**), while phage particles occurred within the completely lysed cells in 8–24 h (**Figure 1c**).

TEM analysis of the phages of *Vibrio* host cells showed the occurrence of tailless phages with a double-layer membrane covering the icosahedral head. Phage-uninfected, phage-infected, and completely lysed *Vibrio* cells are shown (**Figure 1d–f**).

2.10 Antagonism of *Bacillus* against *Vibrio* spp.

An antagonism assay consisting of cell-free extract of *Bacillus* BT21, *Bacillus* BT22, and *B. subtilis* BT23 showed inhibitory activity against several pathogenic species of *Vibrio* [40]. They reported that *B. subtilis* BT23 exhibits a relatively higher inhibitory activity than the other two *Bacillus* BT21 and *Bacillus* BT22. Moreover *B. subtilis* BT23 was shown to inhibit the growth and proliferation of 112 isolates of *Vibrio* spp. consisting of *V. harveyi* (39 isolates), *V. anguillarum* (24 isolates), *V. vulnificus* (30 isolates), and *V. damsela* (19 isolates) which were obtained from *P. monodon* culture hatcheries and ponds. The growth and proliferation of pathogenic *V. harveyi* was inhibited by *B. subtilis* BT23 culture inoculated at a preliminary level of 10^5–10^9 cfu ml^{-1}, whereas lower concentrations of *B. subtilis* BT23 (10^5 and 10^7 cfu ml^{-1}) allowed early growth and proliferation followed by a decrease in the total viable counts of *V. harveyi*. Co-culture experiments demonstrated that the growth and proliferation of *V. harveyi* was controlled under *in vitro* conditions when the concentrations of *B. subtilis* BT23 were increased. Experiments of cell-free

extracts of *Bacillus subtilis* BT23 established the inhibitory effects on the growth and proliferation of *V. harveyi* in liquid culture under aerobic conditions. The inhibitory efficacy was higher in *B. subtilis* BT23 cell-free extracts of 10^8 cfu ml^{-1} and low in 10^4 cfu ml^{-1}. Cell-free extracts of *Bacillus subtilis* BT23 initially could not limit the growth and proliferation of *V. harveyi* for 2 days, and afterward the growth and proliferation of *V. harveyi* was extremely inhibited when compared with the growth of *V. harveyi* without *B. subtilis* BT23. The studies on the probiotic treatment *V. harveyi*-infected *P. monodon* revealed a substantial reduction in the mortality of shrimp which were treated with *B. subtilis* BT23 strains under *in vivo* conditions. The cumulative mortality of *V. harveyi* post-infection and probiotic *B. subtilis* BT23-untreated *P. monodon* was 50% on the ninth day and 100% on the seventeenth day of post-infection. In contrast, in the probiotic treatment groups, the cumulative mortality of shrimp was 10% in the combined treatment after 5 days post-infection. Long-term and short-term treatments of *V. harveyi*-infected *P. monodon* with *B. subtilis* BT23 showed a decrease in cumulative mortality of 32 and 60%, respectively. In control groups of *V. harveyi*-uninfected *P. monodon*, there was no mortality. The growth of pathogenic *V. harveyi* was inhibited by nonpathogenic *B. subtilis* BT23 under *in vivo* and *in vitro* conditions. Co-culture experiments showed that the inhibitory activity of *B. subtilis* BT23 increased with increasing density of the antagonist. A high concentration of *B. subtilis* BT23 (antagonist) was essential to obstruct the growth and multiplication of *V. harveyi* in the co-culture experiments. The antagonist required being present at significantly higher levels than the pathogen, and the degree of inhibition increased with the level of antagonist. During the co-culture, $10^7/10^8$ cfu ml^{-1} was required to inhibit the growth of the pathogen *V. harveyi*. Therefore, a potential probiotic co-culture must either be supplied on a regular basis or be able to colonize and multiply on or in the host. A similar control of pathogenic *Vibrio* in fish and shellfish, by the use of nonpathogenic bacterial strains and disease prevention, has received much attention during the last decade [42, 43, 56, 81]. Purification and characterization of the antibacterial substances from the host bacteria and the phages could help to understand the mechanism of antibacterial activity of *Bacillus* and other strains. Probiotic treatment offers a very promising alternative to the use of antibiotics in fish and shrimp aquaculture. Further study is needed to elucidate the exact mode of action of the observed beneficial effects of the probiotics and to understand the possibilities and limitations of microbial control in aquaculture.

2.10.1 Long-term storage of phages

The usefulness of a phage lysate preparation and treatment method adopted for long-term storage of phages was elucidated in a study that demonstrated the infectivity of the phages of *Vibrio* spp. that remained unaffected with chloroform and DMSO treatments and storage at −40°C for 30 days [37–39, 41]. Similarly, phages were shown to be highly stable under normal storage conditions and also stable in NaCl and $MgSO_4$ due to its stabilizing effect. Substantial amounts of viable phages were reported to occur even after storage even in distilled water. Phage isolates were found to be stable upon storage at 4°C, and a rapid loss of phage infectivity was encountered with repeated freezing and thawing at −70°C. *Bacillus* phage was stable to a 1-hour exposure to chloroform, indicating that it probably does not contain chloroform-soluble lipids and lipoproteins. *V. vulnificus* phage infectivity could not be inhibited with trypsin, protease, and ribonuclease treatments, while the infectivity of the *Vibrio* phages was inhibited with lysozyme and SDS treatments, perhaps demonstrating that the enzyme has interfered with the adsorbing of the phages [38, 39, 41]. The enzymatic treatments and inhibition of phage

infectivity of several phages were reported. Proteinase K treatment could not alter the adsorption ability of phage particles. These studies show that binding of the phages to its host cell membrane is the first step of lytic cycle and this can be an irreversible mechanism, and a similar mechanism may exist in the infection of vibriophages as the infectivity of the phages were inhibited by lysozyme and SDS treatments. Treatment with 1% SDS did not affect the adsorption ability of phage particles. Similarly *Mycoplasma arthritidis* virulent 1 (MAV1) phage infectivity was reported to be unaffected by treatment with Triton X-100 and was resistant to nonionic detergents. *Vibrio* phages were found to be fairly resistant to chloroform [46, 101]. Further studies on the proteins and lipids of *V. vulnificus* phages may help us to understand their role in the life cycle and infectivity of phages. *V. vulnificus* phages survived 100% at pH 7 and exhibited infectivity, while none of the phages survived at extreme pH conditions (pH 3 and pH 12) [41]. *V. harveyi* phages were inactivated at pH 3 or less and at pH 12 or greater. In contrast, VPP97 phage was totally inactivated at pH below 5 or over 10. All *V. vulnificus* phages from the shrimp *Penaeus monodon* exhibited optimal survival at 37°C, but infectivity occurred, and plaques were observed up to a maximum temperature of 50°C [41]. JSF9 phage was shown to be stable at a temperature below 37°C, and the phages were rapidly inactivated above 50°C temperature. *V. parahaemolyticus* phage (VPP97) has been shown to be stable up to a temperature of 65°C and was totally inactivated at 70°C [41, 54, 58, 77, 101]. Phages were reported to survive extremes of temperature up to 95°C [54]. Phages, viz. T-ϕD0, T-ϕD2S, T-ϕHSIC, and T-ϕD1B, exhibited a latent period ranging from 90 to 180 min [77]. The results have clearly shown the physicochemical parameters are very important for the survival and infectivity of phages [37, 38, 46, 49]. Additional studies related to infectivity, stage specific expression of proteins, and specific effects of the purified phage proteins are needed to better understand their functions and applications in the control and process of infectivity of *V. vulnificus* phages.

2.10.2 Phage therapy for vibriosis in shrimp

Phage therapy is a re-emerging field, and the bacteriophages represent potential biocontrol agents for the control of virulent and drug-resistant bacteria [19, 23–73]. However the use of phage therapy in shrimp is still in its early years, and these are highlighted especially the need for using more than one kind of bacteriophage in aquaculture to evade development of bacterial resistance [47–49, 58–60]. Phage therapy has been effectively used to protect against *Vibrio* diseases in a shrimp and prawn hatchery. Inhibitory effects of bacteriophages against shrimp pathogenic *Vibrio* spp., efficacy of potential phage cocktails against *Vibrio harveyi* and closely related *Vibrio* species isolated from shrimp, morphological characterization and biocontrol effects of *Vibrio vulnificus* phages against vibriosis in the shrimp, and a phage therapy in aquaculture system have been described [37–39, 41]. Four new *V. vulnificus* phages were detected from shrimp aquaculture system, named VV1, VV2, VV3, and VV4 [39]. All lytic *V. vulnificus* phages belonged to *Tectiviridae* family with typical double-layered elongated icosahedral head and tailless morphology [39, 101]. Lytic *V. vulnificus* phages which infect other *Vibrio* isolates were further characterized for long-term storage by enzyme treatment, organic solvent treatment, detergent treatment, pH stability, temperature stability, and agar bioassay method and one-step growth experiment. The infectivity, growth, and multiplication of VV1, VV2, VV3, and VV4 phages were unaffected by the treatment effects of chloroform, acetone, ethyl alcohol, methyl alcohol, ribonuclease (RNase), trypsin, protease, and Triton-X100. The phages (VV1–VV4) were inactivated completely with temperature (over 60°C), pH (below 3 and above 12), and

lysozyme and sodium dodecyl sulfate (SDS) treatment. One-step growth experiments indicated a latent period of 3 h and a burst size at 37°C. Agar bioassay method indicated that the percentage inhibition of the bacteria *Vibrio* was 75 (VV1) and 70 (VV2, VV3, and VV4), respectively. *V. vulnificus* phages had a broad lytic spectrum and potential biocontrol of luminous vibriosis in the shrimp aquaculture system [39, 41, 101]. The lytic *Vibrio vulnificus* phages may provide a better understanding of phage-host interactions and development of phage therapy in the aquaculture system. Besides, 12 *V. harveyi* phages showing broad host ranges were recovered from seawater samples. Further, some of the phages of ϕH17-5c, ϕH17-7b, ϕH17-8b, and ϕH17-9b were reported to show inhibitory activity against *V. harveyi* based on various tests, viz. heat stability, chloroform stability, adsorption rate, and one-step growth experiment [58]. A bacteriophage of *Vibrio harveyi* was secluded from shrimp farm water from the West coast of India and demonstrated to exhibit a broad lytic activity against *V. harveyi* isolates [48, 49]. *V. harveyi*-infected larval shrimp exhibited a higher rate of survival in the presence of the bacteriophage than the uninfected control larval shrimp. Bacteriophage treatment of the vibriosis of shrimp in hatchery tanks enhanced the survival of the *V. harveyi*-infected shrimp larvae and reduced the bacterial counts [50–55, 57, 58]. Bacteriophages secluded from a shrimp hatchery and farmed *P. monodon* samples exhibited lytic activity against *V. harveyi* and also controlled the population of *V. harveyi* and improved the survival of *Penaeus monodon* larvae [41, 48–52, 58]. Purified bacteriophages exhibited lytic activity, indicating they are appropriate for phage therapy application [29, 53]. A few bacteriophages that infected *V. harveyi* in a shrimp hatchery were unable to control the outbreak of luminescent vibriosis disease in a shrimp culture system [75]. The potential of the bacteriophages of *Vibrio harveyi* was reported to control population of pathogenic *Vibrio harveyi* in a hatchery setting [48]. In a set of laboratory experiments, post-larval *Penaeus monodon* was exposed to 10^6 cfu ml^{-1} *cells of Vibrio harveyi* and was treated with 100 ppm phage which has led to a drastic reduction of *Vibrio harveyi* counts with 86% survival of the infected larvae, while the survival of the phage-untreated larvae was 25%. In the antibiotic (oxytetracycline 5 ppm, kanamycin 100 ppm/day)-treated hatchery tanks, an initial reduction of luminous bacterial counts was shown, and after 48 h the bacterial count increased to 10^6 ml^{-1}, showing the luminous vibriosis and mortality of the nauplii of *Penaeus monodon* with 40% larval survival. In contrast, in the bacteriophage-treated tank, the larval survival was 86%, and the survival rate in the control tank was only 17% [48]. The presence of *V. mimicus* (15 isolates), exhibiting a typica profile of *Vibrio* [49] (two isolates), was obtained from diseased tissues of penaeid shrimp. A prominent occurrence of *V. harveyi*, *V. furnissii*, *V. mimicus*, *V. damsela*, and *V. anguillarum* in the hatchery tank water besides MBV- and WSSV-uninfected *P. monodon*, sea sediment, seawater, and shrimp culture pond sediment and shrimp culture pond water has been recorded [30, 53]. Vibriosis (*V. alginolyticus*)-associated mortality has been recorded in several invertebrates such as *Penaeus monodon* and *Macrobrachium rosenbergii*. Phage therapy has been shown to inhibit the growth and multiplication of *V. harveyi*, *V. parahaemolyticus*, and *V. anguillarum*. Bacteriophages belonging to *Siphoviridae* family are positive to control *Vibrio* species as the *Siphoviridae* phages are considered to have a specific host range consisting of species of *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio campbellii* [41, 44, 46, 48–53, 58, 60, 69]. A lytic phage PW2 was obtained from shrimp pond water in Songkhla Province, Thailand, and the morphological characteristics of the phage consisted of an icosahedral head and a long noncontractile tail categorized under the order *Caudovirales* and family of *Siphoviridae*. The phage PW2 showed lytic properties against *Vibrio harveyi* [52]. Most of the *Vibrio harveyi* phages were found to be siphophages [25, 26, 29, 41, 51]. However, *Vibrio harveyi*

phages which were from other families such as *Myoviridae* and *Podoviridae* were also reported [41, 51]. Selection of a suitable bacteriophage or a cocktail of phages is the key issue in the success of phage therapy of *Vibrio* species. Moreover phage cocktails have been demonstrated to be more effective than individual phages in treatment of *Vibrio* infection. By making a phage cocktail, it would become easier to treat a wide range of drug-resistant bacterial infections [58, 68]. Moreover phage-resistant mutants are exceptional, and hence the use of a multiphage therapy might decrease the resistance mechanism [61–65, 81, 82]. The limitations of the use of bacteriophages have been recognized, and the phages cannot be used if they (1) have toxin gene insertion; (2) have endotoxin release due to their lytic effect on the host bacterial cells; (3) have a risk of genetic material exchange, i.e., transduction or phage conversion; (4) have propagation and continuous maintenance; (5) have development of anti-bacteriophage bodies against them; (6) have the cost temperate phages contributing to bacterial virulence; (7) have emergence of phageresistant bacteria; (8) have disease outbreak of unknown bacteria when phage specificity is a problem; and (9) are continuously removed by the immune system [80–82]. Vibriophages themselves may have some protective effects though they can be candidates as therapeutic agents in bacterial infections in the aquaculture system, and therefore there is a need to be further investigating them in their natural environment. Future work may involve obtaining consistent credible results which can substantiate the application of bacteriophages to control vibriosis in shrimp and extending such research to other organisms. The usefulness of phage therapy to control microbial infections that occur in dissimilar organisms at various stages of from eggs to brood stock, as well as in laboratory, tanks, or field applications, was shown in experiments made with shrimp larvae, showing a promising potential for phage therapy [46]. The life-saving antibiotics and the new-generation antibiotics cannot be used in aquaculture, and therefore the bacteriophages have the natural advantages and potential to be used in management of the vibriosis in aquaculture.

2.10.3 Phage therapy in lobsters

Significant (71%) mortalities of larval, post-larval, and adult lobsters were caused by shell diseases where *Vibrio* spp., besides *Pseudomonas*, and *Aeromonas* have also been isolated. A strain of *Vibrio owensii* (DY05) was isolated from an epizootic in aquaculture-reared larval phyllosomas of the ornate spiny lobster *Panulirus ornatus* [10–12, 55]. Bacteriophage therapy was one of the techniques that controlled and removed the pathogenic *Vibrio* spp. from the larval cultures of the tropical rock lobster, *Panulirus ornatus*. *V. harveyi* has been found to be associated with diseases in spiny lobster [55].

Crothers-Stomps and colleagues [55] demonstrated that from eight bacteriophages (six phages belonged to the family *Siphoviridae*, and two belonged to the family *Myoviridae*), only one bacteriophage from the family *Siphoviridae* was shown to exhibit a clear lytic activity against *V. harveyi* with no apparent transducing properties. They have identified the occurrence of phage resistance as a major constraint to the use of phage therapy in aquacultures as the pathogenic bacteria were not completely eliminated [29, 41], and a similar approach on other phages is essential for understanding of the mechanism of development of phage resistance.

2.10.4 Diseases of Macrobrachium rosenbergii (DeMan)

Bacterial and fungal diseases are accountable for the high mortality and profound economic loss encountered in the giant freshwater prawn *M. rosenbergii* aquaculture industry [19–21]. Vibriosis (*V. alginolyticus*)-associated mortality was

recorded in *M. rosenbergii* [19–21]. They have shown the occurrence of a very high load of total heterotrophic bacterial count and a total presumptive *Vibrio* count in the 11 different larval stages of *M. rosenbergii*, whereas the total heterotrophic bacterial count was higher in the larval tank water throughout the cycle of the *M. rosenbergii* larval culture. The control treatment methods for the *vibriosis* consisted of chlorination, application of antibiotics, UV radiation alone, and a combination of sequential treatments starting from chlorination (2 ppm) followed by sand filtration, dechlorination, UV radiation (10 s) and microfiltration (5 μm size), and the sequential treatments in *M. rosenbergii*, and these had resulted in the gradual reduction of *V. alginolyticus* counts, and these were shown to be the best methods to control the pathogenic bacterial population in hatcheries of freshwater prawn *M. rosenbergii*. Antimicrobial resistance profile of *Vibrio* species isolated from the hatchery system of *M. rosenbergii* has been reported [19–21]. Vibriosis of *M. rosenbergii* can be controlled through phages φSt2 and φGrn1 of *V. alginolyticus* from live feed *A. salina* [97–99].

2.10.5 Diseases of crabs

Shell diseases are caused by chitinolytic bacteria which were encountered in English prawn *Palaemon serratus*; American lobsters *Homarus americanus*; penaeid shrimp; and king crabs, *Paralithodes camtschaticus* and *Paralithodes platypus*; and the tanner crabs *Chionoecetes tanneri* are affected by rust diseases caused by chitin-destroying bacteria [7]. Biocontrol method against the infection of *V. anguillarum* for rearing the swimming crab larvae *Portunus trituberculatus* in the aquaculture water has been described [94–96]. A bacterial strain PM-4 *Thalassobacter utilis* isolated from a crustacean culturing pond was shown to inhibit the growth of pathogenic *Vibrio anguillarum* in seawater and to improve the growth of larval crab. The cells of the bacterial strain PM-4 *T. utilis* were cultured in a large quantity and were added daily for 6^3 of seawater used for culturing larval crab *Portunus trituberculatus*. Initial *V. anguillarum* bacterial density in the crustacean culture water was 10^6 cells ml^{-1}, while at the crab larval growth stage zoea II, the bacterial density was found to increase to more than 10^7 cells ml^{-1} and reduced the pathogenic *V. anguillarum* bacteria to 10^6 cells ml^{-1}. When the bacterial strain PM-4 *Thalassobacter utilis* dominated the bacterial populations, the numbers of pathogenic bacteria *Vibrio* spp. were reduced or could not be detectable in seawater. The growth and production of the larval crab *P. trituberculatus* were found to be increased by the addition of the bacterial strain PM-4 to the culture water. Investigations on the bacteriophages and phage therapy specific for aquaculture of crabs are desirable.

2.10.6 Phage therapy for vibriosis of Artemia salina

The occurrences of *V. harveyi*, *V. furnissii*, *V. mimicus*, *V. damsela*, and *V. anguillarum* in the nauplii of *Artemia* and the presence of *V. harveyi* and *V. mimicus* in the *Artemia*-reared water have been recorded [12, 18, 22, 39]. A prominent occurrence of *Vibrio* in the *Artemia* nauplii, *Artemia*-reared water, egg samples, hatchery tank water besides MBV- and WSSV-uninfected *P. monodon*, sea sediment, seawater, and shrimp culture pond sediment and shrimp culture pond water was recorded. Vibriosis (*V. alginolyticus*)-associated mortality has been recorded in several invertebrates such as *Penaeus monodon* and *Macrobrachium rosenbergii* [18–21, 34, 97–99]. *Vibrio* species normally reside in the live feed organism such as *Artemia salina* which serves as means of carrying and establishing the bacteria into the hatchery and aquaculture system where *V. alginolyticus* has been reported as the

prevailing member of the cultivable bacterial community of *Artemia*. There are a number of studies indicative that *Artemia* nauplii are vectors of potentially harmful bacteria such as *Vibrio* spp. Goulden et al. [12] reported that *Vibrio owensii* (DY05)-infected *Artemia* (brine shrimp) was responsible for 84–89% mortality of the aquacultured larval phyllosomas of the ornate spiny lobster *Panulirus ornatus*, and an understanding of the infection processes can help to improve targeted biocontrol strategies. The existing disinfection techniques such as filters, chlorination, ozone, UV, etc. could not prevent the occurrence of bacterial pathogens in the hatcheries and may perhaps help in the growth, proliferation, and multiplication of the opportunistic pathogen application of broad-spectrum antibiotics in the feed and hatchery water which has been the most natural strategy to control bacterial infections. The practice of applying antibiotics in aquaculture has become unattractive as many important bacterial pathogens belonging to the genera *Aeromonas* and *Vibrio* evolve antibiotic resistance. The excessive and indiscriminate use of antibiotics has resulted in the development of multidrug-resistant bacterial strains and public health problems. In shrimp hatcheries, the use of bacteriophages that infect bacteria is an alternative method to selectively eliminate their bacterial hosts while leaving normal microbiome unaltered. Phage therapy has been shown to inhibit the growth and multiplication of *V. harveyi*, *V. parahaemolyticus*, and *V. anguillarum* [39, 97–99].

2.10.7 In vitro lytic effect of phages φSt2 and φGrn1 on Vibrio strains of Artemia salina

In vitro cell lysis experiment of phage (a) *φ*St2 and (b) *φ*Grn1 was carried by infecting the fresh cultures of the host bacteria *V. alginolyticus* strain V1 which was collected from live feed *A. salina* culture [97–99]. The lysis of the host bacteria *V. alginolyticus* strain V1 grown in TCBS was proportional to the multiplicity of infection (MOI) (which is defined as the ratio between the number of viruses in an infection and the number of the host cells which can be resolved by correcting the relative concentration of virus and host) used. The lowest (MOI = 1) showed no effect, while the highest (MOI = 100) showed a complete inhibition of bacterial growth. The phages were also tested *in vitro* against 12 different bacterial isolates grown in TCBS which originated from live feed *A. salina* culture. A phage mixture of *φ*St2 and *φ*Grn1 at MOI = 100 was shown to affect the growth of all 12 bacterial strains tested, and the study showed that in all cases, there was a delay in the exponential phase, and even when the cultures reached a plateau of growth, the density of phage-treated bacteria was lower than their corresponding controls.

2.10.8 In vivo efficacy of phages on the vibriosis of A. salina culture

The effect of the phage mixture (*φ*St2 and *φ*Grn1 at MOI = 100) was examined by administrating the phages *in vivo* in the live prey cultures of *A. salina*. After 4 h of administration of the phage mixture, *Vibrio* count was not altered in the phage-untreated control cultures, while the *Vibrio* count drastically reduced in the phage-treated cultures of *A. salina* [97]. The total *Vibrio* counts in the phage-treated cultures of *A. salina* was $5.3 \times 10^3 \pm 3.1 \times 10^3$ cfu ml^{-1}, which was 93% lesser than that of the initial total *Vibrio* counts. The *φ*St2 and *φ*Grn1 phages affected the growth of a range of *Vibrio* spp. and exhibited lytic activity in eight different *V. alginolyticus* strains [97]. The phages also exhibited infection in 10 out of the 25 *Vibrio* strains (40%) tested and lysis in bacterial strains such as *V. harveyi* and *V. parahaemolyticus* species and were known to affect the growth of several strains of bacteria *V. harveyi* strains isolated from an *Artemia* live feed organism. A broad host range of *φ*St2 and *φ*Grn1 phages indicated the occurrences of a very similar host

structures as receptors, viz. LPS phage receptors on the outer membrane of many of Gram-negative bacteria and the genetic similarity contributing to this broad lytic spectrum of the phages φSt2 and φGrn1 phages which exhibited a strong lytic effect against *V. alginolyticus*-type strain DSM 2171 [98]. The bacteriophages, φSt2 and φGrn1, are having a broad host range, and such biological attributes make them the potential candidates for phage therapy application, and these advocate that phage therapy can be an alternative to antibiotics in aquaculture. The phages, viz. φSt2 and φGrn1 and a giant bacteriophage pVa-21, can effectively be used in the biological control of pathogenic *Vibrio* species in marine hatcheries [97–99]. These studies indicate the potential applications of the phages for various purposes outside aquaculture, and further research on the specificity, phage-host interactions, proteomics, and genomics of phages are needed to establish their usefulness and utilization. Efficacy of phage therapy was shown to prevent mortality of the *vibriosis*-infected brine shrimp *Artemia franciscana* [97–99]. Application of single-type/cocktails of phages against *V. parahaemolyticus*- and *V. harveyi*-infected cysts and nauplii *Artemia franciscana* showed a drastic improvement in the survival rate (from 85 to 89%) and hatching success (100% in both cases) in groups treated with phages, whereas the control groups exhibited a survival rate of 40–50% and hatching success (50%). These studies indicate that the phage cocktails offer an alternative to chemotherapeutic agents and can be used in brine shrimp production.

3. Conclusions

The callous use of antibiotics against bacterial diseases in the culture of crustaceans in the aquatic system has led to the development of antibiotic-resistant bacterial infections which can be a serious threat to all life forms and public health, and the phage therapy may help to overcome such complex problems. Control of bacterial diseases in the future may depend on development of novel drugs, innovative approaches, and management practices to minimize the risk of introduction of infectious agents into aquaculture systems and to reduce predisposing factors. The occurrences of lytic bacteriophages of *Bacillus* spp./*Vibrio* spp. as well as their efficacy were established. Phage therapy has been shown to inhibit the growth and multiplication of many different pathogenic bacteria, viz. *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. furnissii*, *V. mimicus*, *V. damsel*, and *V. anguillarum*, and promote better survival and production of the crustaceans in aquaculture. Infected live feed *Artemia* is responsible for the establishment of the bacterial infections in the hatchery and aquaculture system causing mass mortality of the larval crustaceans in culture, and such bacterial infections can be controlled through lytic phages, viz. ϕH17-5c, ϕH17-7b, ϕH17-8b, and ϕH17-9b from *V. harveyi*; phages VV1, VV2, VV3, and VV4 from *V. vulnificus*; and φSt2 and φGrn1 of *V. alginolyticus* of *A. salina*. However development of specific cocktails of phage therapy for aquaculture of crabs, prawns, lobsters, and crabs is desirable. The phage therapy is an ideal method for the control of microbial infections that occur in dissimilar organisms at various stages from eggs to brood stock as well as in laboratory, tanks, or field applications, and extending such research to other organisms could be one of the valuable strategies, to provide evidences and validate the usefulness and therapeutic potential of the phage therapy. An understanding of the infection processes, phage resistance, the efficacy of phage therapy on the targeted pathogens, and their impact on the normal microbiome can help to improve biocontrol strategies. The potential applications of the phages for various purposes outside aquaculture and further research on the specificity and phage-host interactions are needed to establish their usefulness and exploitation. Future work may be

carried out on the intricacies of phage lifestyles and their dynamics in natural systems, genome and viriomes, proteome analysis, genes coding for their proteins, and DNA polymerase phylogeny, which can help us in identifying novel methods of phage-host interaction and in understanding the way in which phages control their hosts. The use of probiotic organisms and phage therapy in crustacean aquaculture is found to be safe for the consumer of shrimp, and therefore a scientific rational approach is needed to evolve guidelines for phage therapy/probiotic applications in aquaculture and for their use and safety.

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Conflict of interest

The author declares that there is no financial or conflict of interests.

Author's contribution

The author (Dr P. Ramasamy) has made a significant contribution to the conception, design, and execution of the reported study and drafted the manuscript writing and discussion.

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References

[1] FAO. The state of world aquaculture. Fisheries Technical Paper; 500, 2006

[2] FAO. The State of World Fisheries and Aquaculture—2008; 2009

[3] The State of World Fisheries and Aquaculture. FAO's Official World Fishery and Aquaculture; 2018

[4] The shrimp panel at the National Fishery Institute's. In: Global Seafood Market Conference (GSMC); Miami, Florida, USA; 21-25 January 2018. 2018

[5] Lundin GG. Fish health quarantine. In: The World Bank Report. Global Attempts to Address Shrimp Disease. Marine/Environmental Paper 4. 1996. p. 45

[6] Stentiford G, Sritunyalucksana K, Flegel TW, Williams BAP. New paradigms to help solve the global aquaculture disease crisis. PLoS Pathogens. 2017;**13**:2

[7] Sindermann CJ. Principal Diseases of Marine Fish and Shellfish. 2nd ed. Vol. 2. United Kingdom, London: Academic Press; 1990

[8] Baross JA, Tester PA, Morita RY. Incidence, microscopy, and etiology of exoskeleton lesions in the tanner crab, *Chionoecetes tanneri*. Journal of the Fisheries Research Board of Canada. 1978;**35**:1141-1149

[9] Taylor CC. Shell disease as a mortality factor in the lobster (*Homarus americanus*). Fish Circ. Maine, Dep. Sea Shore Fish; 1948. pp. 41-48

[10] Malloy SC. Bacteria induced shell disease of lobsters (*Homarus americanus*). Journal of Wildlife Diseases. 1978;**14**:2-10

[11] Roald SO, Aursjø J, Hästein T. Occurrence of shell disease in lobsters, *Homarus gammarus* in the southern part of the OsloFjord. Norway: FiskeriDirektoratets Skrifter Serie HavUndersokelser; 1981;**17**:153-160

[12] Goulden EF, Hall MR, Bourne DG, Pereg LL, Høj L. Pathogenicity and infection cycle of *Vibrio owensii* in larviculture of the ornate spiny lobster (*Panulirus ornatus*). Applied and Environmental Microbiology. 2012; **78**(8):2841-2849. DOI: 10.1128/ AEM.07274-11 Epub 2012 Feb 3

[13] Austin B, Zhang XH. *Vibrio harveyi*: A significant pathogen of marine vertebrates and invertebrates. Letters in Applied Microbiology. 2006;**43**(2): 119-124

[14] Molitoris E, Joseph SW, Krichevsky MI, Sindhuhardja W, Colwell RR. Characterization and distribution of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* isolated in Indonesia. Applied and Environmental Microbiology. 1985;**50**(6):1388-1394. 0099-2240/85/121388-07$02.00/0

[15] Vaseeharan B, Ramasamy P. Abundance of potentially pathogenic micro-organisms in India. *Penaeus monodon* larval rearing hatcheries in India. Microbiological Research. 2003; **158**(4):299-308

[16] Ramasamy P, Rajan PR, Jayakumar R, Rani S, Brennan GP. *Lagenidium callinectus* (Couch, 1942) infections and its control in aquacultured larval (nauplii, zoea and mysis) Indian tiger prawn *Penaeus monodon*. Journal of Fish Diseases. 1996; **19**:75-82

[17] Jayakumar R, Ramasamy P. Bacterial and protozoan (ciliate) diseases of prawn *Penaeus indicus* (*Crustacea*). Indian Journal of Marine Sciences. 1999;**28**:285-296

[18] Krishnika A, Ramasamy P. Effect of water exchange to eliminate *Vibrio* sp.

during the naupliar development of *Artemiafranciscana*. Journal of Fisheries and Aquatic Science. 2012; **7**(3):205-214

[19] Krishnika A, Ramasamy P. Antimicrobial resistance profile of *Vibrio* species isolated from the hatchery system of *Macrobrachium rosenbergii* (Deman). Indian Journal of Fisheries. 2014;**60**(4):147-152

[20] Krishnika A, Ramasamy P. Effect of chlorination, antibiotics, and UV radiation of *Vibrio* population in the hatchery system of *Macrobrachium rosenbergii* (DeMan). Asian Journal of Microbiology, Biotechnology & Environmental Sciences. 2013;**15**(1): 105-112

[21] Krishnika A, Ramasamy P. *Lagenidium* sp infection in the larval stages of the fresh water prawn *Macrobrachium rosenbergii* (DeMan). Indian Journal of Fisheries. 2014;**61**(2): 90-96

[22] Karunasagar I, Karunasagar I, Umesha RK. Microbial diseases in shrimp aquaculture. In: Ramaiah N, editor. Marine Microbiology: Facets and Opportunities. Goa: National Institute of Oceanography; 2000. pp. 121-134

[23] Nakai T, Sugimoto R, Park K-H, et al. Protective effects of bacteriophage on experimental *Lactococcus garvieae* infection in yellowtail. Diseases of Aquatic Organisms. 1999;**37**:33-41

[24] Børsheim KY. Native marine bacteriophages. FEMS Microbiology Ecology. 1993;**102**:141-159

[25] Thiel K. Old dogma, new tricks—21st century phage therapy. Nature Biotechnology. 2004;**1**:31-36

[26] Marks T, Sharp R. Review: Bacteriophages and biotechnology: A review. Journal of Chemical Technology and Biotechnology. 2000;**75**:6-17

[27] Heldal M, Bratbak G. Production and decay of viruses in aquatic environments. Marine Ecology Progress Series. 1991;**72**:205-212

[28] Fuhrman JA, Suttle CA. Viruses in marine planktonic systems. Oceanography. 1993;**6**:51-63

[29] Nakai T, Park SC. Bacteriophage therapy of infectious diseases in aquaculture. Research in Microbiology. 2002;**153**(1):13-18

[30] Withey S, Cartmell E, Avery LM, et al. Bacteriophages—Potential for application in wastewater treatment processes. Science of the Total Environment. 2005;**339**(1-3):1-18

[31] Levin BR, Bull JJ. Population and evolutionary dynamics of phage therapy. Nature Reviews. Microbiology. 2004;**2**(2):166-173

[32] Chanishvili N. Phage therapy—History from twort and D'Herelle through soviet experience to current approaches. Advances in Virus Research. 2012;**83**:4-40. DOI: 10.1016/B978-0-12-394438-2.00001-3

[33] D'Hérelle F. Surun microbe invisible antagoniste des *Bacillus* dysentérique. Comptes rendus de l'Académie des Sciences-Series D. 1917;**165**:373-375

[34] Sulakvelidze A, Kutter E. Bacteriophage therapy in humans. In: Kutter E, Sulakvelidze A, editors. Bacteriophages: Biology and Application. Boca Raton: CRC Press; 2005. pp. 381-436

[35] Whitman WB et al. Prokaryotes: The unseen majority. Proceedings of the National Academy of Sciences. 1998;**95**: 6578-6583

[36] Ramasamy P, Brennan GP. Phage therapy in aquaculture system. In: Trends in Biotechnology. India: University of Madras, Publication Division; 2002. pp. 53-60

[37] Srinivasan P, Vaseeharan B, Ramasamy P. *Vibrio* bacteriophages control the growth of bacterial populations in the aquatic environment. In: The Sixth Indian Fisheries Forum organized by Central Institute of Fisheries Education, Versova, Mumbai —400 061, India; during 17–20 December 2002; 16

[38] Srinivasan P, Ramasamy P. Effect of pH, temperature, enzymes, organic solvents and detergents in the survival and infectivity of *Vibrio* bacteriophages. In: Conference on Microbiology of the Tropical Seas (COMITS) National Institute of Oceanography; Goa, India during 13–15 December 2004

[39] Srinivasan P, Ramasamy P, Brennan GP, et al. Inhibitory effects of bacteriophages on the growth of *Vibrio* sp. pathogens of shrimp in the Indian aquaculture environment. Asian Journal of Animal and Veterinary. 2007;**2**(4): 166-183

[40] Vaseeharan B, Ramasamy P. Control of pathogenic *Vibrio* spp. *Bacillus ubtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*. Letters in Applied Microbiology. 2003;**36**(2):83-87

[41] Srinivasan P, Ramasamy P. Morphological characterization and biocontrol effects of *Vibrio vulnificus* phages against *Vibriosis* in the shrimp aquaculture environment. Microbial Pathogenesis. 2017;**111**:472-480

[42] Park SC, Shimamura I, Fukunaga M, et al. Isolation of bacteriophages specific to a fish pathogen, *Pseudomonas plecoglossicida*, as a candidate for disease control. Applied and Environmental Microbiology. 2000;**66**(4):1416-1422

[43] Park SC, Nakai T. Bacteriophage control of *Pseudomonas plecoglossicida* infection in ayu *Plecoglossus altivelis*. Diseases of Aquatic Organisms. 2003;**53**: 33-39

[44] O'Flaherty S, Ross RP, Coffey A. Bacteriophage and their lysins for elimination of infectious bacteria. FEMS Microbiology Reviews. 2009;**33**(4): 801-819

[45] Kim JH, Gomez DK, Nakai T, et al. Isolation and identification of bacteriophages infecting ayu *Plecoglossus altivelisaltivelis* specific *Flavobacterium psychrophilum*. Veterinary Microbiology. 2010;**140**(1-2):109-115

[46] Karunasagar I, Vinod MG, Kennedy B, et al. Biocontrol of bacterial pathogens in aquaculture with emphasis on phage therapy. In: Walker PJ, Lester RG, Bondad-Reantaso MG, editors. Diseases in Asian Aquaculture V. Fish Health Section. Manila: Asian Fisheries Society; 2005. pp. 535-542

[47] Austin B, Stuckey LF, Robertson PAW, Effendi I, DRW G. A control agents in aquaculture. Journal of Ocean University of China. 1995;**6**(1): 76-79

[48] Vinod MG, Shivu MM, Umesha KR, et al. Isolation of *Vibrio harveyi* bacteriophage with a potential for biocontrol of luminous vibriosis in hatchery environments. Aquaculture. 2006;**255**(1-4):117-124

[49] Karunasagar I, Shivu MM, Girisha SK, et al. Biocontrol of pathogens in shrimp *Hatcheries* using bacteriophages. Aquaculture. 2007;**268** (1-4):288-292

[50] Chrisolite B, Thiyagarajan S, Alavandi SV, et al. Distribution of luminescent *Vibrio harveyi* and their bacteriophages in a commercial shrimp hatchery in SouthIndia. Aquaculture. 2008;**275**(1-4):13-19

[51] Shivu MM, Rajeeva BC, Girisha SK, et al. Molecular characterization of *Vibrio harveyi* bacterio-phages isolated from aquaculture environments along

the coast of India. Environmental Microbiology. 2007;**9**(2):322-331

[52] Phumkhachorn P, Rattanachaikunsopon P. Isolation and partial characterization of a bacteriophage infecting the shrimp pathogen *Vibrio harveyi*. African Journal of Microbiology Research. 2010;**4**: 1794-1800

[53] Maeda M, Liao IC. Effect of bacterial population on the growth of a shrimp larva, *Penaeus monodon*. Bulletin of National Research Institute of Aquaculture (Japan). 1992;**21**:25-29

[54] Jiang SC, Kellogg CA, Paul JH. Characterization of marine temperate phage-host systems isolated from Mamala Bay, Oahu, Hawaii. Applied and Environmental Microbiology. 1998; **64**:535-542

[55] Crothers-Stomps C, Høj L, Bourne DG, et al. Isolation of lytic bacteriophage against *Vibrio harveyi*. Journal of Applied Microbiology. 2010; **108**(5):1744-1750

[56] Wu JL, Lin HM, Jan L, Hsu YL, Chang LH. Biological control of fish bacterial pathogen, *Aeromonas* hydrophila by bacteriophage AH 1. Fish Pathology. 1981;**15**:271-276

[57] Rong R, Lin H, Wang J, Khan MN, Li M. Reductions of *Vibrio parahaemolyticus* in oysters after bacteriophage application during depuration. Aquaculture. 2014; **418-419**:171-176

[58] Kalatzis PG, Bastías R, Kokkari C, Katharios P. Isolation and characterization of two lytic bacteriophages, φSt2 and φGrn1; phage therapy application for biological control of *Vibrio alginolyticus* in aquaculture live feeds. PLoS One. 2016;**11**(3):e0151101. DOI: 10.1371/journal.pone.0151101. eCollection 2016

[59] Plaza N, Castillo D, Pérez Reytor D, Higuera G, García K, Bastías R. Bacteriophages in the control of pathogenic vibrios. Electronic Journal of Biotechnology. 2018;**31**:24-33. DOI: 10.1016/j.ejbt.2017.10.012

[60] Kim SG, Jun JW, Giri SS, Yun S, Kim HJ, Kim SW, et al. Isolation and characterisation of pVa-21, a giant bacteriophage with anti-biofilm potential against *Vibrio alginolyticus*. Scientific Reports. 2019;**9**:6284

[61] Reardon S. Phage therapy gets revitalized. The rise of antibiotic resistance rekindles interest in a century-old virus treatment. Nature. 2014;**510**:15-16

[62] Sulakvelidze A, Alavidze Z, Morris JG Jr. Bacteriophage therapy. Antimicrobial Agents and Chemotherapy. 2001;**45**(3):649-659

[63] Srinivasan P, Ramasamy P. Occurrence, distribution and antibiotic resistance patterns of *Vibrio* species associated with viral diseased shrimp of south Indian aquaculture environment. International Journal of Agriculture Sciences. 2009;**1**(2):1-10

[64] Gemmell CG, Edwards DI, Fraise AP, Gould FK, Ridgway GL, Warren RE, et al. Guidelines for the prophylaxis and treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the UK. Journal of Antimicrobial Chemotherapy. 2006;**57**(4):589-608

[65] Lodise TP, McKinnon PS. Burden of methicillin-resistant *Staphylococcus aureus*: Focus on clinical and economic outcomes. Pharmacotherapy. 2007;**27**: 1001-1012

[66] Międzybrodzki R, Fortuna W, Weber-Dąbrowska B, Górski A. Phage therapy of staphylococcal infections (including MRSA) may be less expensive than antibiotic treatment.

Postepy Hig Med Dosw. 2007;**61**: 461-465

[67] Smith HW, Huggins MB. Successful treatment of experimental *Escherichia coli* infections in mice using phage: Its general superiority over antibiotics. Journal of General Microbiology. 1982;**128**: 307-318

[68] Holmström K, Gräslund S, Wahlström A, et al. Antibiotic use in shrimp farming and implications for environmental impacts and human health. International Journal of Food Science and Technology. 2003;**38**(3): 255-266

[69] Karunasagar I, Pai R, Malathi GR, et al. Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. Aquaculture. 1994;**128** (3-4):203-209

[70] Jayakumar R, Ramasamy P. Prevalence, biotyping and resistotyping of *Pseudomonas* spp. and *Vibrio* sp isolated from *Penaeus indicus* of Ennore Estuary, Madras, India. In: Chou LM, Munro AD, Lam TJ, Chen TW, Cheong LKK, Ding JK, et al. editors. Proceedings of Third Asian Fisheries Forum, Singapore. 1994. pp. 335-338

[71] Vaseeharan B, Ramasamy P, Murugan T, Chen JC. *In vitro* susceptibility of antibiotics against *Vibrio* spp. and *Aeromonas* spp. isolated from *Penaeus monodon* hatcheries and ponds. International Journal of Antimicrobial Agents. 2005; **26**:285-291

[72] Ramasamy P, Sujatha Rani J, Gunasekaran DR. Assessment of antibiotic sensitivity and pathogenicity of *Vibrio* spp. and *Aeromonas* spp. from aquaculture environment. MOJ Ecology & Environmental Sciences. 2018;**3**(3): 128-136

[73] Vaseeharana B, Lin J, Ramasamy P. Effect of probiotics, antibiotic sensitivity, pathogenicity, and plasmid profiles of *Listonella* anguillarum-like bacteria isolated from *Penaeus monodon* culture systems. Aquaculture. 2004;**241**: 77-91

[74] Verschuere L, Rombaut G, Sorgeloos P, et al. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews. 2000; **64**(4):655-671

[75] Rengipipat S, Rukpratanporn S, Piyatiratitivorakul S, Menasaveta P. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). Aquaculture. 2000;**191**:271-288

[76] Gatesoupe FJ. The use of probiotics in aquaculture. Aquaculture. 1999;**180** (1-2):147-165

[77] Adamek Z. Effect of ascogen probiotics supplementation on the growth rate of rainbow trout *Oncorhynchus mykiss* under conditions of intensive culture. Zivocisna Vyroba. 1994;**39**(3):247-253

[78] Balcázar JL, De Blas I, Ruiz-Zarzuela I, Cunningham D, Vendrell D, Múzquiz JL. The role of probiotics in aquaculture. Veterinary Microbiology. 2006;**114**:173-186. DOI: 10.1016/j. vetmic.2006.01.009

[79] Van Hai N, Buller N, Fotedar R. The use of customised probiotics in the cultivation of western king prawns (*Penaeus latisulcatus* Kishinouye, 1896). Fish & Shellfish Immunology. 2009;**27**: 100-104

[80] Nayak SK. Probiotics and immunity: A fish perspective. Fish & Shellfish Immunology. 2010;**29**(1):2-14

[81] Magnadottir B. Immunological control of fish diseases. Marine

Biotechnology (New York, N.Y.). 2010; **12**(4):361-379

[82] Chapman CM, Gibson GR, Rowland I. Health benefits of probiotics: Are mixtures more effective than single strains? European Journal of Nutrition. 2011;**50**(1):1-17

[83] Fjellheim AJ, Klinkenberg G, Skjermo J, Aasen IM, Vadstein O. Selection of candidate probionts by two different screening strategies from Atlantic cod (*Gadusmorhua* L.) larvae. Veterinary Microbiology. 2010;**144**: 153-159

[84] Heidarieh M, Afsharnasab M, Soltani M, Dashtyannasab A, Rajabifar S, Sheikhzadeh N, et al. Effects of ergosan and vibromax to prevent vibriosis and WSSV in *Litopenaeus vannamei*. Journal of Fisheries and Aquatic Science. 2010;**5**: 120-125

[85] Fuller R. History and development of probiotics. In: Probiotics. Netherlands: Springer; 1992. pp. 1-8

[86] Robles R, Sorgeloos P, Van Duffel H, Nelis H. Progress in biomedication using live foods. Journal of Applied Ichthyology. 1998;**14**: 207-212

[87] Green A, Green M. Probiotics in Asian shrimp aquaculture. MPEDA Newsletter; 2003

[88] Haung HJ. Important tools to the success of shrimp aquaculture—Aeration and the applications of tea seed cake and probiotics. Aquaculture International. 2003:13-16

[89] Ahilan B. Probiotics in aquaculture. Aquaculture International. 2003:39-40

[90] Mishra S, Mohanty S, Pattnaik P, Ayyappan S. Probiotics—Possible role in aquaculture. Fishing Chimes. 2001; **21**(1):31-36

[91] Jameson JD. Role of probiotics in aquaculture practices. Fishing Chimes. 2003;**23**:9

[92] Shakibazadeh S, Saad CR, Christianus A, Kamarudin MS, Sijam K, Sinaian P. Assessment of possible human risk of probiotic application in shrimp farming. International Food Research Journal. 2011;**18**:433-437

[93] Kumar V, Roy S, Meena DK, Sarkar UK. Application of probiotics in shrimp aquaculture: Importance, mechanisms of action, and methods of administration. Reviews in Fisheries Science & Aquaculture. 2016;**24**(4): 342-368. DOI: 10.1080/ 23308249.2016.1193841

[94] Nogami K, Maeda M. Bacteria as biocontrol agents for rearing larvae of the crab *Portunus trituberculatus*. Canadian Journal of Fisheries and Aquatic Sciences. 2011;**49**(11): 2373-2376. DOI: 10.1139/f92-261

[95] Nogami K, Hamasaki K, Maeda M, Hirayama K. Biocontrol method in aquaculture for rearing the swimming crab larvae *Portunus trituberculatus*. Hydrobiologia. 1997;**358**:291-295

[96] Nogami K, Hamasaki K, Maeda M, Hirayama K. Biocontrol method in aquaculture for rearing the swimming crab larvae *Portunus trituberculatus*. In: Hagiwara A, Snell TW, Lubzens E, Tamaru CS, editors. Live Food in Aquaculture. Developments in Hydrobiology. Vol. 124. Dordrecht: Springer; 1997

[97] Martínez-Díaz SF, Hipólito-Morales A. Efficacy of phage therapy to prevent mortality during the vibriosis of brine shrimp. Aquaculture. 2013;**400-401**: 120-124. DOI: 10.1016/j. aquaculture.2013.03.007

[98] Quiroz-Guzmán E, Peña-Rodriguez A, Vázquez-Juárez R, Barajas-Sandoval DR, Balcázar JL, Martínez-Díaz SF.

Bacteriophage cocktails as an environmentally-friendly approach to prevent *Vibrio parahaemolyticus* and *Vibrio harveyi* infections in brine shrimp (*Artemia franciscana*) production. Aquaculture. 2018:273-279. DOI: 10.1016/j.aquaculture.2018.04.025

[99] Quiroz Guzman E, Peña A, Vázquez-Juárez R, Martínez-Díaz SF. Bacteriophage cocktails as an environmentally-friendly approach to prevent *Vibrio parahaemolyticus* and *Vibrio harveyi* infections in brine shrimp (*Artemia franciscana*) production. Aquaculture. 2018;**492**:273-279

[100] Oakey HJ, Cullen BR, Owens L. The complete nucleotide sequence of the *Vibrio harveyi bacteriophage* VHML. Journal of Applied Microbiology. 2002; **93**(6):1089-1098

[101] Stalin N, Srinivasan P. Characterization of *Vibrio parahaemolyticus* and its specific phage from shrimp pond in Palk Strait, South East coast of India. Biologicals. 2016; **44**(6):1-8. DOI: 10.1016/j.biologicals.2016.08.003

Section 5

Bioaccumulation

Chapter 10

Assessment of Metal Accumulation and Bioaccumulation Factor of Some Trace and Heavy Metals in Freshwater Prawn and Crab

Osikemekha Anthony Anani and John Ovie Olomukoro

Abstract

Globally, freshwater decapods have been one of the major food delicacies because of their rich deposits of minerals. High metals are usually accumulated in the body tissues of these organisms because of their lifestyle. Metal accumulation in freshwater decapods has been acclaimed and perceived to cause serious health concerns when transferred to humans along the food chain. A recent study has shown that freshwater biota, prawn (*Macrobrachium rosenbergii*), showed significant differences ($p < 0.05$) in Mn, Cu, Pb and Cr and no significant difference ($p > 0.05$) in Fe, Zn and Cd. In contrast, the freshwater biota, crab (*Sudanonautes africanus*), showed significant differences ($p < 0.05$) in Fe, Zn and Mn and no significant differences ($p > 0.05$) in Pb, Cr and Cd. A high accumulation of Fe in the whole tissues of *Macrobrachium rosenbergii* and *Sudanonautes africanus* was also established. This is because Fe in the Nigerian soil and sediment is naturally very high beyond slated thresholds and tend to accumulate and transcend or magnify in benthic. It was noticed that Zn (2.68) and Cr (4.52) had the highest bioaccumulation factors in prawn and crab, respectively. Chromium has been observed to be carcinogenic. The consumption of Cr in the muscles of crab might constitute probable serious health risk.

Keywords: accumulation, biomagnification, decapods, risk, carcinogenicity

1. Introduction

Globally, freshwater decapods have been one of the major food delicacies because of their rich deposits of minerals, metals; calcium (Ca), iron (Fe), zinc (Zn) and copper (Cu), Nickel (Ni), Vanadium (V) and Cadmium (Cd) as well as nutrients; protein, fibers and cellulose. High levels of mineral contents like metals are usually accumulated in the body tissues of these organisms because of their lifestyle. This has necessitated the increased rate of human consumption in recent times [1].

It has been documented that freshwater crab (*Sudanonautes africanus*) and prawn (*Macrobrachium rosenbergii*) have high deposits of Fe, Zn and Mn, with few traces of Cu, Pb, Cr, Cd, Ni and V [1–4].

Metal accumulation in freshwater decapods has been acclaimed and perceived to cause serious health concerns when transferred to humans along the food chain. Environmental valuation of the noxiousness of metals in the freshwater *Sudanonautes africanus* and *Macrobrachium rosenbergii* had been shown to have probable human health hazard effect concomitant by way of ingestion [1].

Health risks associated with heavy metals such as renal failure, skeletal deformation, and hepatic failure have been linked to their non-decomposable and persistence nature in the visceral organ-parts of humans [5]. This can lead to severe maladies like dysentery, stomach aches, head-tremor, anemia, paralysis, nausea, paroxysm, melancholy and even respiratory disorders [6], which can be either acute or chronic forms; neuron toxicity, oncogenic, genetic alteration or teratogenicity [7].

High levels of bio-accumulated heavy metals in freshwater decapods have been identified in these ranks; Fe > Zn > Mn > Cu > Pb > Cr [1] as interconnected with their sediment background levels [8].

2. Methodology

2.1 Sampling technique

Samples of freshwater prawns and crabs (*Macrobrachium rosenbergii* and *Sudanonautes africanus*) were captured and collected monthly from March 2015 to August 2016 at designated stations by some fishermen, using local-hand nets enticed with ox-heart, set at the river bank of each station 24 hours before capturing. The prawns and crabs were collected and kept in different labeled plastic rubbers according to the stations, filled with the river water and immediately taken to the laboratory for heavy metal analysis and identifications.

2.2 Extraction and determination of heavy metals in freshwater decapods

Employing the methods of [1], the freshwater biota was oven dried at 105°C. About 2 g of a dried up standardized sample of each tissue were digested and sample was made up to about 50 ml of purified water. Samples of the biota were analyzed for iron, manganese, zinc, copper, chromium, cadmium, nickel, lead, and vanadium with an AAS (atomic absorption spectrophotometer; SOLAAR 969AA UNICAM, Spectronic Unicam, Cambridge, UK) [1].

2.3 Data analysis

Simple descriptive analysis, ANOVA (Analysis of variance), was employed using SPSS version 20.0.

To determine the accumulation rate of heavy metals in the freshwater biota, the bio-indices; bioaccumulation factor was employed with Micro Excel version 2013. Bioaccumulation factor (BAF) is the concentration of metals in sediment or water over the concentration of metals in the biota in mg kg^{-1} [8]. This can be represented in the equation below:

$$\mathrm{BAF} = \frac{\text{concentration of metals in sediment/water (mg/kg)}}{\text{concentration of metals in Crab/prawn (mg/kg)}} \quad (1)$$

3. Assessment of metal contents in freshwater decapods

The assessment of metal contents in freshwater decapods is suitable for both water and terrestrial life forms. This is based on their significance as water indicators of pollutants via monitoring in spatial or temporal, in order to quantify their ecological role in the ecosystem. Even though the dangers of water pollution by metals are fully acknowledged, it is still a subject of discussing in line with the over increasing anthropogenic activities [9] and as well as the lithogenic activities [1].

Freshwater prawn and shrimp (Macroinvertebrates) are commonly recommended as fauna-indicators for evaluating the fluctuation of aquatic disorders in the region of probable pollution [3]. In general, decapods are of certain prominence for bio-monitoring survey [10], as the bedrock species in most aquatic systems [11], as well as the most tolerated species against water pollution. This shows their pollution status as regards to the buildup of the corresponding components of metals in their muscles [12].

3.1 Accumulation of metals in *Sudanonautes africanus* and *Macrobrachium rosenbergii*

Metal contamination in freshwater ecosystem is of utmost worry everywhere in the biosphere [13–15]. This might be as a result of their persistence noxious special effects and accumulation features in the aquatic system and fauna respectively [16–22].

Metals go in into freshwater ecosystem via lithogenic and human activities [18, 22–24]. Benthic region is a key sink and a basis for metal pollution [25]. Built-up substances such as metals can be a sign of macrobenthic fauna-accumulation severity, ill health or death as the case may arise [24]. Certain residential chemicals in the sediment can exterminate benthic macroinvertebrates, thus plummeting the food chain structure [26].

However, a recent study has shown that freshwater biota; prawn (*Macrobrachium rosenbergii*) showed significant differences ($p < 0.05$) in Mn, Cu, Pb and Cr and no significant difference ($p > 0.05$) in Fe, Zn and Cd. No observable p-values were noticed for Ni and V respectively (**Table 1**). In contrast, the freshwater biota; crab (*Sudanonautes africanus*) showed significant differences ($p < 0.05$) in Fe, Zn and Mn and no significant difference ($p > 0.05$) was observed in Pb, Cr and Cd. There was also no observed p-values in Ni and V (**Table 2**) [28]. It was noticed that the ranks of heavy metals and their spatial variability in the shrimps and crabs were Fe > Zn > Cu > Pb = Cd > Cr = Ni = V and Fe > Zn > Mn > Cu > Pb > Cr = Cd > Ni = V respectively.

3.2 Bioaccumulation

The food chain structure has served as a pointer where metals are streamed along. Freshwater ecosystem pollution by metals, especially the heavy ones is on the increase daily around the world, causing several problems globally. Consequent of the buildup effect of certain heavy metals, particularly via the food chain, their bio-availability needs to be examined. This can be done via investigation of metal contents in biota in order to gather and predict its bioavailability and subsequent accumulation in the organism(s).

Generally, freshwater decapods freely accumulate metals in their muscles in order to meet their basic metabolic needs. This makes them appropriate as bio-indicators of metals in the ecosystem. For example, freshwater *Sudanonautes africanus* and *Macrobrachium rosenbergii* accumulate high levels of Fe, Zn and Mn in their muscles [28] based on the facts that these metals play vital role in the respiratory pigment

Parameters	Units	Station 1	Station 2	Station 3	Station 4	p-values	Significant	[27] limits
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD			
Fe	mg kg^{-1}	120.99 ± 43.95	150.45 ± 71.66	148.48 ± 86.88	119.16 ± 49.36	0.25	$p > 0.05$	100
Zn	mg kg^{-1}	45.82 ± 22.46	52.13 ± 23.26	57.79 ± 21.14	53.06 ± 18.55	0.32	$p > 0.05$	100
Mn	mg kg^{-1}	1.61 ± 1.41	1.94 ± 1.16	2.08 ± 1.68	1.13 ± 0.46	0.04	$p < 0.05$	1.0
Cu	mg kg^{-1}	0.39 ± 0.32	0.73 ± 0.48	0.72 ± 0.57	0.66 ± 0.32	0.04	$p < 0.05$	30
Pb	mg kg^{-1}	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.02	$p < 0.05$	0.5
Cr	mg kg^{-1}	0.00 ± 0.00	0.01 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.05	$p < 0.05$	NS
Cd	mg kg^{-1}	0.01 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.01 ± 0.01	0.62	$p > 0.05$	0.5
Ni	mg kg^{-1}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	—	NS
V	mg kg^{-1}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	—	NS

Most of the parameters were measured in mg/kg; $p < 0.05$, significant difference; $p > 0.05$, no significant difference. NS, not specified; FAO, Food and Agriculture Organization; WHO, World Health Organization.

Table 1.
Summary of the accumulation of heavy metals in prawns from Ossiomo River collected from designated stations from March 2015 to August 2016.

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Parameters	Units	Station 1	Station 2	Station 3	Station 4	p-values	Significance	[27] limits
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD			
Fe	mg kg^{-1}	154.55 ± 41.40	203.42 ± 76.29	167.86 ± 118.00	170.28 ± 113.14	0.02	$p < 0.05$	100
Zn	mg kg^{-1}	66.59 ± 21.15	92.99 ± 31.40	66.80 ± 51.76	69.73 ± 50.92	0.02	$p < 0.05$	100
Mn	mg kg^{-1}	1.98 ± 1.60	3.68 ± 2.59	3.31 ± 2.95	3.57 ± 3.11	0.02	$p < 0.05$	1.0
Cu	mg kg^{-1}	0.64 ± 0.27	1.13 ± 0.74	0.88 ± 0.78	0.95 ± 0.81	0.03	$p < 0.05$	30
Pb	mg kg^{-1}	0.01 ± 0.02	0.03 ± 0.04	0.03 ± 0.04	0.04 ± 0.04	0.19	$p > 0.05$	0.5
Cr	mg kg^{-1}	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.03	0.34	$p > 0.05$	NS
Cd	mg kg^{-1}	0.00 ± 0.01	0.02 ± 0.03	0.02 ± 0.03	0.02 ± 0.03	0.23	$p > 0.05$	0.5
Ni	mg kg^{-1}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	—	NS
V	mg kg^{-1}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	—	NS

Most of the parameters were measured in mg/kg; $p < 0.05$, significant difference; $p > 0.05$, no significant difference. NS, not specified; FAO, Food and Agriculture Organization; WHO, World Health Organization.

Table 2.
Summary of the accumulation of heavy metals in crab from Ossiomo River collected from designated stations from March 2015 to August 2016.

hemocyanin [29] and metalloenzymes [30] respectively. An appreciable increase in the values of Mn in the whole tissue of freshwater decapods has been associated with a combination of factors such as co-factor [1, 30–32]. More so, high concentration of iron in the sediment and decapods can possibly be related to its presence in cytochromes and proteins [28, 32].

A high accumulation of Fe in the whole tissues of *Macrobrachium rosenbergii* and *Sudanonautes africanus* has been established by [28]. This is because Fe in the Nigerian soil and sediment is naturally very high beyond slated thresholds and tends to accumulate and transcend or magnify in benthic macroinvertebrates [24, 33–35].

Freshwater fauna are well known to be discriminatory in metal accumulation [36]. Antón et al. and Hopkin [37, 38], stated that decapods regulate their net assimilation of metals, which need about 0.07 mg kg^{-1} of Zn and 0.08 mg kg^{-1} of Cu to trigger the enzymes and respiratory proteins. The high levels of heavy metals in aquatic biotas are of particular interest because of the potential risk to humans who consume them [1, 36]. The effects of metals in the surroundings, rest on to a great magnitude on whether they exist in forms that can be assimilated by plants or animals [36]. Some freshwater decapods are bottom feeders and are generally expected to concentrate more metals than surface feeders like shrimp. The accumulation of metals in their muscles may be either dosage or time-reliant. This may therefore be contemplative of the amount of metals in the ecosystem [37–41].

3.3 Bioaccumulation factor: designated trace and heavy metals and impact in freshwater *Macrobrachium rosenbergii* and *Sudanonautes africanus*

Table 3 shows the computed bioaccumulation factor for the different trace and heavy metals in the whole body tissue of freshwater prawn and crab. It was observed that the concentration of the BAFs of heavy metals of prawn as compared with that of crab was distinct with varied increase in values greater than 1 (BAFs > 1) Fe (2.68) in prawn and Fe, Zn, Mn, Cu, Pb and Cr (1.30, 1.45, 1.77, 1.41, 1.81, and 4.52) in crab as related to their sediment concentrations. It was noticed that Cr had the highest value of accumulation in crab.

Trace and heavy metal in mgkg^{-1}	Mean Trace and heavy metal in Sediment	Mean Trace and heavy metal in Shrimps	Mean Trace and heavy metal in Crabs	BAF in Shrimps	BAF in Crabs
Fe	249.11	134.77	175.27	**0.54**	1.30
Zn	19.46	52.20	75.46	2.68	1.45
Mn	36.53	1.69	2.99	**0.05**	1.77
Cu	8.07	0.63	0.88	**0.08**	1.41
Pb	3.48	0.01	0.02	**0.00**	1.81
Cr	4.05	0.00	0.01	**0.00**	4.52
Cd	3.93	0.01	0.01	**0.00**	1.06
Ni	1.98	0.00	0.00	**0.00**	**0.00**
V	1.72	0.00	0.00	**0.00**	**0.00**

NB: Values in red; means BAF > 1, an indication of increase level beyond threshold. Black bolded values; means BAF < 1, an indication of safe limit.

Table 3.
Results of bioaccumulation factors (BAFs) of trace and heavy metals in freshwater prawn (Macrobrachium rosenbergii) and crab (Sudanonautes africanus) in Ossiomo River.

3.3.1 Iron (Fe)

Iron, is the richest element in the Earth's crust [42]. The two oxidation states of Fe; ferrous (Fe^{2+}), and ferric (Fe^{3+}) account for their Fenton chemical reactions in aquatic fauna via combination with their macromolecules (proteins, nucleic acids, lipids and carbohydrates) [43]. On the other hand, ferric iron is virtually insoluble in aqueous solution and can bioaccumulated in freshwater fauna (decapods) in their tissues [44] and even biomagnified along the food chain thereby impeding the health status of humans.

Iron is very vital to quite a lot of life processes; manufacturing of DNA, the respiratory electron transport chain, as well as oxygen storage and transport. However, level of Fe beyond the threshold in fauna muscles can result to conjunctivitis, choroids, and retinitis [45] pneumoconiosis, called siderosis [46] and the risk of pulmonary cancer when ingested or inhaled by humans [43].

3.3.2 Zinc (Zn)

Zinc is one of the essential trace metals in nature. Aquatic fauna depends on it for their survival. Zinc is made up of about 200 metalloenzymes and other metabolic components guaranteeing permanency of the DNA and its assemblies; nuclear membranes, nucleolus and protein structures (ribosomes) [41]. Excessive consumption of Zn can result to a diverse compulsive health impact on humans [47].

The composition of zinc found in the tissue of decapods has been investigated to be intrinsically high which will possibly biomaginify in tissues at much higher levels [48–52]. Possible impact of Zn toxicity in freshwater decapods is in the gills, and abdominal muscle which has been confirmed in juvenile of decapods [53, 54]. This might be basically linked to the comparatively greater and more pervious body nature of the juveniles [41].

3.3.3 Manganese (Mn)

This is a crucial trace metal which can be seriously noxious upon persistent contact through ingestion above threshold limits. The basic dietary requirements of Mn are fulfilled through food intake [55–57], but with little noxious effects from air and water. This is a great concern to individuals who will consume freshwater of *Macrobrachium rosenbergii* and *Sudanonautes africanus* with elevated amounts of Mn accumulated in them.

Possible conditions linked to Mn toxicity are schizophrenia, dreariness, weak brute force, head tremor and sleeplessness [58, 59]. Chronic impacts of Mn are hepatopancreas, lung, liver and vascular instabilities, deteriorations in body fluid pressure, failure in growth of fauna fetuses and brain impairment.

3.3.4 Copper (Cu)

Like manganese, copper is found naturally in the surroundings and also crucial for normal growth and metabolic rate of all fauna [60] especially the aquatic ones. Copper contributes immensely to the cellular metalloprotein-hemocyanin in freshwater decapods [49, 61]. However, fairly low copper contents are found in the muscles of freshwater *Sudanonautes africanus* and *Macrobrachium rosenbergii* [28].

Freshwater *Sudanonautes africanus* and *Macrobrachium rosenbergii* cannot be compared to their counterpart; crayfish, which is very valuable for evaluating bioavailability of Cu in water environments [62, 63].

3.3.5 Lead (Pb)

This element is neither crucial nor valuable to aquatic fauna and causes series of health conditions in the biota [40, 60] and subsequently probable risk impact to man via the food chain [1]. Pb can be introduced into a freshwater ecosystem via lithogenic form; re-suspension of the bottom sediment by benthic dwellers [1, 24] or via anthropogenic inputs; fertilizers and pesticides.

The amount of lead accumulated in freshwater *Sudanonautes africanus* and *Macrobrachium rosenbergii* has been investigated to be fairly below the benchmark limits of [27]. Previous studies have stated that the amount of Pb found in muscles of decapods were also in line of the benchmark limit [54, 64–67]. Contrary, [68] observed a high concentration (0.15 mg l^{-1}) of Pb in the gonads of the freshwater crab, *Potamonautes perlatus*.

At low concentrations, Pb may result to a variety of health effects, including behavioral problems and learning disabilities [58]. Lead affects the central and peripheral nervous systems, eventually causing neurological and behavioral disorders in patients [69]. Lead has been found to be carcinogenic and also a probable enzyme stimulating effect [70], which interferes with fertility and causes renal damage.

3.3.6 Chromium (Cr)

This is a crucial element that has high noxious level [71]. Chromium has been found to be very high in the muscles of certain decapods [41, 54–67, 72]. However, study on freshwater *Sudanonautes africanus* and *Macrobrachium rosenbergii* revealed fairly low amount of Cr in their whole tissue [28].

3.3.7 Cadmium (Cd)

This is not an essential element and has high potential for teratogenicity, cancer-causing, and high latency for kidney toxicity at the chronic stage if ingested via food [72, 73].

Bioaccumulation of residue Cd in aquatic ecosystems and decapods whole tissue have been described to have a positive relationship consequent on the biota closeness to point source [9, 74–78].

3.3.8 Nickel (Ni) and vanadium (V)

Nickel and vanadium are universal elements recognized for their noxiousness, persistence, and likeness for bio-accumulation [60]. However, they were below detectable limits (BDL) in *Sudanonautes africanus* and *Macrobrachium rosenbergii* in this study [28]. This might be as a result of the geo-formation of the ecosystem and lack of the use of Ni and V related materials around the study terrain.

4. Conclusions

The assessment of metal accumulation and bioaccumulation factor of some trace and heavy metals in freshwater prawn and crab (*Sudanonautes africanus* and *Macrobrachium rosenbergii*) have shown that the metal accumulation were in this ranks: Fe > Zn > Cu > Pb = Cd > Cr = Ni = V and Fe > Zn > Mn > Cu > Pb > Cr = C d > Ni = V. The BAFs values obtained were observed to be greater than 1 (BAFs > 1) for Fe (2.68) in prawn and also for Fe, Zn, Mn, Cu, Pb and Cr (1.30, 1.45, 1.77, 1.41, 1.81, and 4.52) in crab as related to their sediment concentrations. It was noticed

that Zn and Cr had the highest bioaccumulation factors in prawn and crab respectively. Chromium has been observed to be carcinogenic. Consumption of Cr in the muscles of crab might constitute probable serious health risk.

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Conflict of interest

We declare no conflict of interest.

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References

[1] Anani OA, Olomukoro JO. Health risk from the consumption of freshwater prawn and crab exposed to heavy metals in a Tropical river, Southern Nigeria. Journal of Heavy Metal Toxicity and Diseases. 2018;**3**(2):5. DOI: 10.21767/2473-6457.10024

[2] Storelli MM. Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). Food and Chemical Toxicology. 2008;**46**:2782-2788

[3] Li XY, Liu LJ, Wang YG, Luo GP, Chen X, et al. Heavy metal contamination of urban soil in an old industrial city (Shenyang) in Northeast China. Geoderma. 2013;**192**:50-58

[4] Bogdanovic T, Ujevic I, Sedak M, Listeš E, Šimat V, et al. As, Cd, Hg and Pb in four edible shellfish species from breeding and harvesting areas along the eastern Adriatic coast, Croatia. Food Chemistry. 2014;**146**:197-203

[5] Duruibe JO, Ogwuegbu MDC, Egwurugwu JN. Heavy metal pollution and human biotoxic effects. International Journal of Physical Science. 2007;**2**:112-118

[6] McCluggage D. Heavy Metal Poisoning. The Bird Hospital, Columbia: NCS Magazine; 1991

[7] European Union. Heavy Metals in Wastes. Brussels: European Commission on Environment; 2002

[8] Demina LL, Galkin SV, Shumilin EN. Bioaccumulation of some trace elements in the biota of hydrothermal fields of the Guaymas Basin (Gulf of California). Boletín de la Sociedad Geológica Mexicana. 2009;**61**(1):31-45

[9] Besser JM, Brumbaugh WG, May TW, Schmitt CJ. Biomonitoring of lead, zinc, and cadmium in streams draining lead-mining and non-mining areas, Southeast Missouri, USA. Environmental Monitoring and Assessment. 2007;**129**(1-3):227-241

[10] Schilderman PAEL, Moonen EJC, Maas LM, Welle I, Kleinjans JCS. Use of crayfish in biomonitoring studies of environmental pollution of the river Meuse. Ecotoxicology and Environmental Safety. 1999;**44**(3):241-252

[11] Holdich DM. Distribution of crayfish in Europe and some adjoining countries. Bulletin Français de la Pêche et de la Pisciculture. 2002;**367**:611-650

[12] Suarez-Serrano AC, Alcaraz C, Ibáñez C, Trobajo R, Barata C. Procambarus clarkii as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. Ecotoxicology and Environmental Safety. 2010;**73**(3):280-286

[13] Allen HE. Metal Contaminated Aquatic Sediments. Michigan: Ann Arbor Press; 1995

[14] Guevara R, Rizzo A, Sanchez R. Heavy metal inputs in northern Patagonia lakes from short sediment core analysis. Journal of Radioanalytical and Nuclear Chemistry. 2005;**265**:481-493

[15] Mashal K, Salahat M, Al-Qinna M, Al-Degs Y. Spatial distribution of cadmium concentrations in street dust in an arid environment. Arabian Journal of Geosciences. 2014;**8**(5):3171-3182. DOI: 10.1007/s12517-014-1367-1

[16] Sin SN, Chua H, Lo W, Ng LM. Assessment of heavy metal cations in sediments of Shing Mun River, Hong

Kong. Environment International. 2001;**26**:297-301

[17] Lafabrie C, Pergent G, Kantin R, Pergent-Martini C, Gonzalez JL. Trace metals assessment in water, sediment, mussel and seagrass species-validation of the use of *Posidonia oceanica* as a metal biomonitor. Chemosphere. 2007;**68**:2033-2039

[18] Al-Taani AA, Batayneh A, El-Radaideh N, Al-Momani I, Rawabdeh A. Monitoring of selenium concentrations in major springs of Yarmouk Basin, north Jordan. World Applied Sciences Journal. 2012;**18**:704-714

[19] Barakat A, Baghdadi M, Rais J, Nadem S. Assessment of heavy metals in surface sediments of Day River at Beni-Mellal Region Morocco. Research Journal of Environmental and Earth Sciences. 2012;**4**(8):797-806

[20] Al-Taani AA. Trend analysis in water quality of Al-Wehda Dam, NW Jordan. Environmental Monitoring and Assessment. 2014;**186**:6223-6239

[21] Moosavian SM, Baghernabavi SM, Zallaghi E, Rouzbahani MM, Panah EH, Dashtestani M. Measurement of pollution level caused by heavy metal on vanadium, nickel, lead and copper using bivalves shells of *Timoclea imbricate* species on Bahrakan coast in spring 2013. Jundishapur Journal of Health Sciences. 2014;**6**(4):23473

[22] Al-Taani AA, Batayneh AT, El-Rasaideh N, Ghrefat H, Zumlot T, Al-Rawabdeh AM, et al. Spatial distribution and pollution assessment of trace metal in surface sediments of Ziqlab Reservoir. Environmental Monitoring and Assessment. 2015;**187**:32. DOI: 10.1007/s 10661-015-4289-9

[23] Bai J, Cui B, Chen B, Zhang K, Deng W, Gao H. Spatial distribution and ecological risk assessment of heavy metals in surface sediments from a typical plateau lake wetland, China. Ecological Modelling. 2011;**222**:301-306

[24] Anani OA, JO O. The evaluation of heavy metal load in benthic sediment using some pollution indices in Ossiomo River, Benin City, Nigeria. FUNAI Journal of Science and Technology. 2017;**3**(2):103-119

[25] Li XD, Thornton I. Chemical partitioning of trace and major elements in soils contamination by mining and smelting activities. Applied Geochemistry. 2001;**16**:1693-1706

[26] Pan Y, Brugam RB. Human disturbance and tropic status changes in Crystal Lake, Mc Henry County, Illinois, U.S.A. Journal of Paleolimnology. 1997;**17**:369-376

[27] FAO/WHO. Evaluation of certain food additives and the contaminants mercury, lead and cadmium. In: WHO Technical Report Series. 1989. p. 505

[28] Anani OA. Heavy metal residues and contamination profiles of water, sediment, *Macrobrachium rosenbergii* and *Sudanonautes africanus* in Ossiomo River, Edo State [PhD thesis]. Benin City, Nigeria: University of Benin; 2017. pp. 165-182

[29] Van de Brock WLF. Season levels of chlorinated hydrocarbons and heavy metals in fish and brown shrimps from the Medway Estuary Kent. Environmental Pollution. 1979;**19**:21-28

[30] De Silva SS, Anderson TA. Fish Nutrition in Aquaculture. 1st ed. London: Chapman and Hall; 1995

[31] Murphy PA, Atchison GJ, Mchitosh AW, Kolar DJ. Cadmium and zinc content of fish from an industrially contaminated Lake. Journal of Fish Biology. 1978;**13**:327-335

[32] Enuneku AA, Ezenwa IM, Adeosun S. Bioaccumulation profile of selected heavy metals in whole tissue of *Macrobrachium macrobrachion* and *Macrobrachium vollenhovenii* from Benin River in Delta State, Nigeria. African Scientist. 2016;**17**(1):1-8. Available from: http://www.niseb.org/afs

[33] Kakulu SE. Heavy metals in the Niger Delta: Impact of petroleum industry on the baseline levels [PhD thesis]. Ibadan: University of Ibadan; 1985

[34] Kakulu SE, Osibanjo O, Ajayi SO. Trace metal content of fish and shellfishes of the Niger delta area of Nigeria. Environment International. 1987;**13**:247-251

[35] Edema U, Egborge ABM. Heavy metal content of *Macrobrachim* sp. (Crustacea: Palaemonidae) from Warri River, Nigeria. Tropical Journal of Environmental Science and Health. 1999;**2**(1):65-70

[36] Enuneku AA, Ezemonye LI, Ainerua MO. Human health risk assessment of metal contamination through consumption of *Sesarma angolense* and *Macrobrachium macrobrachion* from Benin River Nigeria. European International Journal of Science and Technology. 1987;**3**:6

[37] Antón A, Serrano T, Angulo E, Ferrero G, Rallo A. The use of two species of crayfish as environmental quality sentinels: The relationship between heavy metal content, cell and tissue biomarkers and physico-chemical characteristics of the environment. The Science of the Total Environment. 2000;**247**:239-251

[38] Hopkin SP. Ecophysiology of Metals in Terrestrial Invertebrates. Pollution Monitoring Series. London: Elsevier Press; 1989

[39] Alcorlo P, Otero M, Crehuet M, Baltanás A, Montes C. The use of the red swamp crayfish (*Procambarus clarkii*, Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the river Guadiamar (SW, Spain). The Science of the Total Environment. 2006;**366**:380-390

[40] Allert AL, Fairchild JF, DiStefano RJ, Schmitt CJ, Brumbaugh WG, Besser JM. Ecological effects of lead mining on Ozark streams: In-situ toxicity to woodland crayfish (*Orconectes hylas*). Ecotoxicology and Environmental Safety. 2009;**72**:1207-1219

[41] Antonín K, Miloš B, Pavel K. Bioaccumulation and effects of heavy metals in crayfish: A review. Water, Air, and Soil Pollution. 2010;**211**:5-16. DOI: 10.1007/s11270-009-0273-8

[42] Aisen P, Enns C, Wessling-Resnick M. Chemistry and biology of eukaryotic iron metabolism. International Journal of Biochemistry and Cell Biology. 2001;**33**:940-959

[43] Hentze MW, Muckenthaler MU, Andrews NC. Balancing acts: Molecular control of mammalian iron metabolism. Cell. 2004;**117**:285-297

[44] Aisen P, Listowsky I. Iron transport and storage proteins. Annual Review of Biochemistry. 1980;**49**:357-393

[45] Braun V, Killmann H. Bacterial solutions to the iron-supply problem. Trends in Biochemical Sciences. 1999;**24**:104-109

[46] Archibald F. *Lactobacillus Planteruna*, an organism not requiring iron. FEMS Microbiology Letters. 1983;**19**:29-32

[47] Eisler R. Zinc hazards to fish, wildlife, and invertebrates: A synoptic review. In: U.S. Fish and Wildlife Service Biological Report. 1993. p. 10

[48] Bryan GW. Zinc regulation in the freshwater crayfish (including

some comparative copper analyses). The Journal of Experimental Biology. 1967;**46**:281-296

[49] White SL, Rainbow PS. Regulation and accumulation of copper, zinc and cadmium by the shrimp *Palaemon elegans*. Marine Ecology Progress Series. 1982;**8**:95-101

[50] White SL, Rainbow PS. Regulation of zinc concentration by *Palaemon elegans* (Crustacea: Decapoda): Zinc flux and effects of temperature, zinc concentration and moulting. Marine Ecology Progress Series. 1984;**16**:135-147

[51] Bagatto G, Alikhan MA. Zinc, iron, manganese and magnesium accumulation in crayfish populations near copper-nickel smelters at Sudbury, Ontario, Canada. Bulletin of Environmental Contamination and Toxicology. 1987c;**38**:1076-1081

[52] Vijayram K, Geraldine P. Are the heavy metals cadmium and zinc regulated in freshwater prawns? Ecotoxicology and Environmental Safety. 1996;**34**:180-183

[53] Bennet-Chambers MG, Knott B. Does the freshwater crayfish *Cherax tenuimanus* (Smith) [Decapoda: Parastacidae] regulate tissues zinc concentrations? Freshwater Crayfish. 2002;**13**:405-423

[54] Bruno G, Volpe MG, De Luise G, Paolucci M. Detection of heavy metals in farmed *Cherax destructor*. Bulletin Français de la Pêche et de la Pisciculture. 2006;**380-381**:1341-1349

[55] US EPA. Health Assessment Document for Manganese. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. Research Triangle Park. NC.EPA 600/8-83-013F; 1984

[56] Francis A, Forsyth C. Toxicity Profile of Manganese [Internet]. 2005. Available from: http://rais.ornl.gov/tox/profiles/mn.shtml [Accessed: February 2011]

[57] Hoekman T. Heavy Metal Toxicology [Internet]. 2008. Available from: www.ucs.mun.ca/~thoekman [Accessed: February 2011]

[58] Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Cadmium. ATSDR/TP-88/08. Atlanta: ATSDR/ U.S. Public Health Service; 1989

[59] Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Nickel. US Department of Health and Human Services, Atlanta, GA, USA. Atyaephyra *desomeresti mesopotamica* (Al-Adub, 1987). Marina Mesopotamica. 2005;**17**(2):365-376

[60] Eisler R. Copper hazards to fish, wildlife, and invertebrates: A synoptic review. In: U.S. Geological Survey. Biological Science Report No. USGS/ BRD/BSR-1997-0002. 1998

[61] Rainbow PS. Trace metal concentrations in aquatic invertebrates: Why and so what? Environmental Pollution. 2002;**120**:497-507

[62] Naqvi SM, Devalraju I, Naqvi NH. Copper bioaccumulation and depuration by red swamp crayfish, *Procambarus clarkii*. Bulletin of Environmental Contamination and Toxicology. 1998;**61**:65-71

[63] Guner U. Freshwater crayfish *Astacus leptodactylus* (Eschscholtz, 1823) accumulates and depurates copper. Environmental Monitoring and Assessment. 2007;**133**:365-369

[64] Roldan BM, Shivers RR. The uptake and storage of iron and lead in cells of the crayfish (*Orconectes propinquus*) hepatopancreas and antennal gland. Comparative Biochemistry and Physiology. C. 1987;**86**:201-214

[65] Meyer W, Kretschmer M, Hoffmann A, Harisch G. Biochemical and histochemical observations on effects of low-level heavy metal load (lead, cadmium) in different organ systems of the freshwater crayfish, *Astacus astacus* L. (Crustacea: Decapoda). Ecotoxicology and Environmental Safety. 1991;**21**:137-156

[66] Anderson MB, Preslan JE, Jolibois L, Bollinger JE, George WJ. Bioaccumulation of lead nitrate in red swamp crayfish (*Procambarus clarkii*). Journal of Hazardous Materials. 1997;**54**:15-29

[67] Mackevičienė G. Bioaccumulation of heavy metals in noble crayfish (*Astacus astacus* L.) tissues under aquaculture conditions. Ekologia. 2002;**2**:79-82

[68] Reinecke AJ, Snyman RG, Nel JAJ. Uptake and distribution of lead (Pb) and cadmium (Cd) in the freshwater crab, *Potamonautes perlatus* (Crustacea) in the Eerste River South Africa. Water, Air, and Soil Pollution. 2003;**145**:395-408

[69] WHO/SDE/WSH. Lead in Drinking-Water Background Document for Development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/03.04/09/Rev/1. Geneva: World Health Organization; 2011

[70] Greenberg AE, Clesceri LS, Eaton AT. Standard Methods for the Examination of Water and Wastewater. 18th ed. Washington: American Public Health Association; 1992. pp. 490-596

[71] Eisler R. Chromium hazards to fish, wildlife, and invertebrates: A synoptic review. In: U.S. Fish and Wildlife Service. Biological Report 85(1.2). 1986. p. 85

[72] Jorhem L, Engman J, Sundström B, Thim AM. Trace elements in crayfish: Regional differences and changes induced by cooking. Archives of Environmental Contamination and Toxicology. 1994;**26**:137-142

[73] Eisler R. Cadmium hazards to fish, wildlife, and invertebrates: A synoptic review. In: U.S. Fish and Wildlife Service. Biological Report 85(1.2). 1985

[74] Anderson RV, Vinikour WS, Brower JE. The distribution of Cd, Cu, Pb and Zn in the biota of two freshwater sites with different trace metal inputs. Holarctic Ecology. 1978;**1**:377-384

[75] Giesy JP, Bowling JW, Kania HJ. Cadmium and zinc accumulation and elimination by freshwater crayfish. Archives of Environmental Contamination and Toxicology. 1980;**9**:683-697

[76] Bagatto G, Alikhan MA. Copper, cadmium and nickel accumulation in crayfish populations near copper-nickel smelters at Sudbury, Ontario, Canada. Bulletin of Environmental Contamination and Toxicology. 1987a;**38**:540-545

[77] Devi M, Thomas DA, Barber JT, Fingerman M. Accumulation and physiological and biochemical effects of cadmium in a simple aquatic food chain. Ecotoxicology and Environmental Safety. 1996;**33**:38-43

[78] Schmitt CJ, Brumbaugh WG, Linder GL, Hinck JE. A screening-level assessment of lead, cadmium, and zinc in fish and crayfish from North-Eastern Oklahoma, USA. Environmental Geochemistry and Health. 2006;**28**:445-471

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This book is divided into five sections. The first section is about Biology and Ecology, and includes the following chapters: “Crustacean”, ”The robber crab Birgus latro (Linnaeus, 1767)”, “Scyllarid lobster biology and ecology”, “Management of the interaction and cannibalism of postlarvae and adults of the freshwater shrimp Cryphiops caementarius (Molina, 1782)”, “Bateman gradients and alternative mating strategies in a marine isopod”, and “The habitat types of freshwater prawns (Palaemonidae: Macrobrachium) with abbreviated larval development in Mesoamerica (Mexico, Guatemala & Belize)”. The second section, Fisheries, includes a chapter on the “Estimation of the maximum sustainable yield and the optimal fishing effort of the blue crab (Callinectes sapidus, Rathbun 1896) of Laguna Madre, Tamaulipas, Mexico”. The third section, Genetics, covers “A comparison of genetic variation in two endemic thermal spring isopods, Thermosphaeroma thermophilum and T. milleri (Crustacea: Isopoda: Sphaeromatidae)”. In the fourth section, Diseases, the chapter is “Phage therapy for control of bacterial diseases of crustaceans”. The fifth section, Bioaccumulation, provides information on “Assessment of metal accumulation and bioaccumulation factor of some trace and heavy metals in freshwater prawn and crab”. The book can be used by students, professors, and researchers in areas related to biological sciences.

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