

E. Parmentier · P. Vandewalle

Further insight on carapid—holothuroid relationships

Received: 23 December 2003 / Accepted: 8 September 2004 / Published online: 20 October 2004
© Springer-Verlag 2004

Abstract Carapidae (or pearlfish) are eel-like fishes that live inside different invertebrates, such as holothurians, sea stars, or bivalves. Those of the genus *Carapus* are commensal and use their host as a shelter, while *Encheliophis* species are parasitic and eat the host's gonads. In areas where they live in sympatry, *C. boraborensis*, *C. homei*, *C. mourlani* and *E. gracilis* are able to inhabit the same host species. Infestation is considered as monospecific when several conspecifics are observed in the same host. However, many aspects of this particular relation remain obscure, e.g. communication between carapids and the defence systems of the different protagonists (carapids and hosts). Experiments have been conducted in the field and laboratory to investigate several aspects of the carapids' relationships with their hosts. Sampling carried out in the Bay of Opunohu (Moorea, French Polynesia) determined the sex ratio of *C. boraborensis* (3:1) and *C. homei* (1:1) and their distribution rate within different Echinodermata. Our study showed that neither species was capable of determining whether a heterospecific already occupied a sea cucumber or not. They were, however, able to locate the sea cucumber's cloaca, due to the excurrent resulting from respiration. The sea cucumber's defence system (Cuverian tubules) minimises predator attacks, but is not effective against carapid intrusion. The Carapidae defence system is twofold. Due to a passive system related to the sea cucumber's low cloacal position, the Cuverian tubules are not expelled when fish enter the cloaca. Moreover, carapids resist sea cucumber toxins better than other reef fish. Their increased resistance might be related to their gills rather than to their mucus coating; however,

the latter may assist the fish in resisting the sticky substances emitted by the Cuverian tubules.

Introduction

Coral reefs have a remarkable biodiversity, with numerous examples of associations between animals. One of the best known examples involves clownfish (*Amphiprion* sp., Pomacentridae) that are able to swim among sea anemone tentacles without being stung by the nematocyst of their host (e.g. Mader 1987; Bauchot 1992; Elliott and Mariscal 1996).

Carapidae (Ophidiiforme), known as pearlfish, offer another remarkable example. Those of the Carapini tribe (*Carapus* and *Encheliophis*) are small, eel-like fishes that live inside different invertebrates (Trott 1970). *Carapus* species are commensal, and use their host as a shelter (Trott and Trott 1972; Trott 1981; Van Den Spiegel and Jangoux 1989; Parmentier et al. 2003). *Carapus* spp. are usually found in the respiratory trees or inside the body cavity of different holothurian species (Arnold 1956; Smith 1964; Shen and Yeh 1987). They are also found in the body cavity of sea stars (Trott and Trott 1972; Meyer-Rochow 1979) and ascidians (Weber 1913). *C. dubius* finds shelter between the shell and the mantle of different bivalves (Castro-Aguirre et al. 1996; Parmentier et al. 2000), while *Encheliophis* species are parasitic and live in the body cavity of various holothurian and sea star species, where they primarily feed off the host's gonads (Cheney 1973; Murdy and Cowan 1980; Parmentier and Vandewalle 2003). In areas of the Pacific Ocean, where they live in sympatry (Markle and Olney 1990), *C. boraborensis*, *C. homei*, *C. mourlani* and *E. gracilis* can inhabit the same host species. Infestation is considered to be monospecific when several conspecifics are observed in the same host (Smith 1964; Branch 1969; Trott 1981; Van Den Spiegel and Jangoux 1989).

Entry into a host has been described for *C. acus* (Emery 1880; Arnold 1953; Gustato 1976), but these

Communicated by S.A. Poulet, Roscoff

E. Parmentier (✉) · P. Vandewalle
Laboratoire de Morphologie Fonctionnelle et Evolutive, Institut de Chimie, Bât. B6, Université de Liège, 4000 Liège, Belgium
E-mail: E.Parmentier@ulg.ac.be
Fax: +32-4-3665024

authors do not agree on the intervening stimuli initiating this behaviour. The fish trying to enter a holothurian swims with its head facing downwards, exploring various objects. Once a host is found, the fish tries to identify the posterior end by swimming along the tegument of the holothurian. According to Emery (1880), the cloaca is located by the excurrent coming from the sea cucumber's respiratory trees. Arnold (1957) and Gustato (1976) postulate that this current does not elicit any response. Once the cloaca is found, one can observe one of two different strategies (Arnold 1953; Gustato et al. 1979; Kloss and Pfeiffer 2000). The fish can either penetrate into the host, headfirst, propelling itself by violent strokes of the tail, or place its head at the entry to the host's anus and bring its thin caudal extremity forwards alongside the fish's body at the level of the lateral line. This latter position allows the fish to use its body as a guide to rapidly bring the tail towards the cloaca. Once the caudal extremity of the fish is inside the holothurian, the carapid redresses itself and enters the host with backwards movements (Kloss and Pfeiffer 2000; Parmentier and Vandewalle 2003). In adults, this mode of entry is used 80% of the time (Gustato et al. 1979). *C. bermudensis* and *E. gracilis* are able to locate the holothurian mainly from its odour, whereas sight plays a minor role (Aronson and Mosher 1951; Trott 1970; Van Meter and Ache 1974).

Except for penetration behaviour, many aspects of the relation between the Carapini and their hosts and among Carapini remain obscure:

1. The host functions as a shelter for the Carapini and serves as a prey source for *Encheliophis* spp., in particular. The host may also, however, provide a favourable place for other kinds of behaviour, such as reproduction.
2. If several fish species are able to live within the same host species, they could be equipped with a means of communication, enabling them either to avoid or to seek each other.
3. The sea cucumber may have a defence system against Carapidae. Different holothurian species are known to expel Cuvierian tubules through the anus. These tubules contain different toxins that rapidly immobilise most organisms with which they come into contact (Nigrelli 1952; Bakus 1968; Van Den Spiegel and Jangoux 1987; Habermelh and Krebs 1990; Kalinin et al. 1996). However, these toxins do not seem to affect carapids, so these fish might also have a defence system.

This study examines the three above-stated aspects. A broad sampling in the Bay of Opunohu (Moorea, French Polynesia) should allow observations concerning which holothurians are the preferred hosts and should allow testing of whether the distribution is mono- or multispecific. To complete these observations, several experiments were conducted in the field and laboratory to verify whether carapids were able to determine if a potential host was already inhabited or not, either by a

conspecific or a heterospecific. Other laboratory experiments were aimed at testing the response of the holothurian in the presence of fish and the reaction of the fish to the release of Cuvierian tubules. *C. boraborensis* and *C. homei* were used in this study, because they are usually found in the same host species (*Bohadschia argus* or *Thelenota ananas*, for example), at the same depth and in similar waters (Parmentier et al. 2002; Parmentier and Vandewalle 2003).

Comparison of the data resulting from these experiments should facilitate the aim of this study: to better understand different aspects of the relationship between *Carapus* spp. and their hosts.

Materials and methods

The studies were conducted in two phases: from July 2000 through August 2000 and from June 2002 through August 2002. Sampling was conducted using SCUBA at 18 stations, varying between 1 and 20 m depth in Opunohu Bay (and in the surrounding lagoon), Moorea, French Polynesia (Fig. 1). All echinoderms collected (the holothurians *Bohadschia argus*, *Thelenota ananas*, *Holothuria fuscogilva*, *Thelenota anax*, *Holothuria nobilis* and the sea star *Calcita novaeguineae*) were examined for the presence of fishes, once back in the laboratory. Each holothurian was slit lengthwise (through the cloaca), each sea star was opened laterally, and both groups of echinoderms were checked for the presence of pearlfish. All specimens of *C. boraborensis* and *C. homei* found were measured to the nearest millimetre with a vernier calliper or with a slat, and sex was determined. The two species were placed in separate aquariums (0.8 m×0.35 m×0.5 m), with running seawater, for

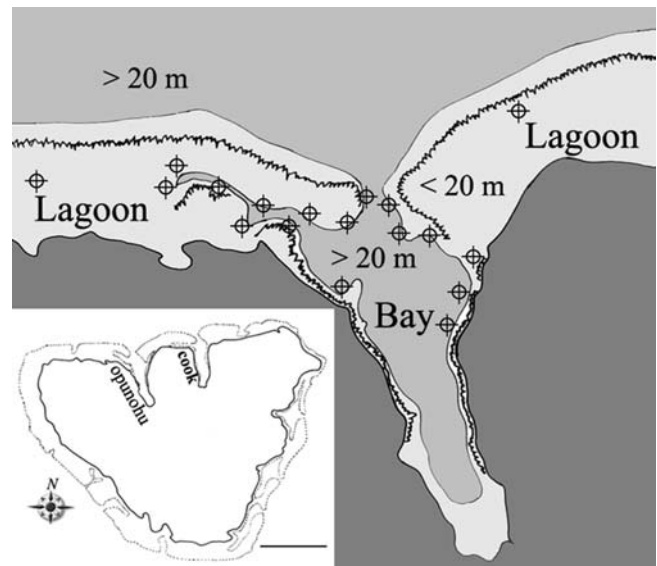


Fig. 1 Map of Opunohu Bay, Moorea Island; showing locations (circles) of the sampling stations on the reef. Scale bar: 16 km

3–5 days before the experiments. Neither fish nor host holothurians were fed during this period.

Four experiments were conducted to see whether the fish were able to determine if a host was already inhabited. An additional goal was to observe the response of heterospecific fishes sharing the same host.

Experiment A

Six *B. argus* were placed in a circular 850-l tank (diameter: 1.5 m; height: 0.7 m) with running seawater. A small 18-l aquarium (0.3 m×0.2 m×0.2 m) containing four *C. boraborensis* and four *C. homei* was placed in the centre of the circular tank, and the eight fishes were released simultaneously. In a second experiment, five *C. boraborensis* and five *C. homei* were used. In both experiments, each fish was directly observed in order to note which sea cucumber it entered. At the end of the experiment, it was then checked if the sea cucumbers had already been occupied.

Experiment B

This experiment allowed observation of cohabiting carapid heterospecifics. It required using uninhabited holothurians (*B. argus*). Prior to the experiment, the holothurians were placed in sealed plastic bags in an aquarium. The sea cucumbers thus respired in a closed space, which led to a depletion of oxygen, causing the fish to leave. Only those holothurians that lost a fish were used in the experiments.

Seven *C. boraborensis* and seven *C. homei* were marked with India ink using a syringe. Two to three small black points were injected in the dermis at different easily recognisable points (opercle, hyomandibular, before the dorsal fin, etc.). A specimen of each species was then placed in an aquarium with a heterospecific and a holothurian; both specimens entered the host within an hour. The seven sea cucumbers sheltering these couples were then simultaneously placed in a tank (diameter = 1.5 m, height = 0.7 m). The entire experiment was conducted twice. The tank was inspected four times a day, and the holothurians were opened after 10 days to remove the carapids.

Experiment C

A Y-tube was constructed for the two-choice experiments (Fig. 2). The central part of the tube was 0.6-m long and had a cross section of 25 cm². The two branches were 30-cm long and were separated transversely into two by a perforated opaque wall (Fig. 2). The aim of these tests was to see if fish are able to choose between two paths. In the first experiment, a branch was connected to an aquarium filled with filtered seawater, and the other to an aquarium containing a holothurian

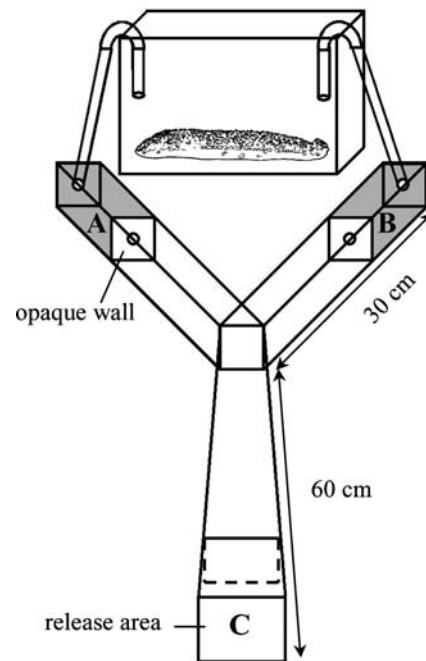


Fig. 2 Schematic illustration of the Y-tube (A, B, C different sections)

(*B. argus*). In the second experiment, the two branches were connected to the same aquarium containing a holothurian (*B. argus*). For the second part, a specimen of *C. boraborensis* was placed behind the opaque wall of a branch (A, Fig. 2) and a specimen of *C. homei* was placed behind the opaque wall of the other branch (B, Fig. 2). The fish to be tested (a total of 17 *C. homei* and 46 *C. boraborensis*) was introduced in the posterior end of the principal branch (C, Fig. 2), waiting 3 min prior to release. We noted the fish's position 5 min post-release or until the fish contacted one of the opaque walls in the upper part of the Y. The choice was analysed by the χ^2 probability test (95% confidence degree, 2 degrees of freedom). The hypothesis according to which the distribution is not random is thus accepted if χ^2 is > 5.99 (Scherrer 1984).

The holothurian was placed in the aquarium 2 h prior to each set of experiments, and the holothurian was changed after approximately ten fish were tested. No one fish was tested more than once daily, or more than two times in all.

Experiment D

Ten holothurians (*B. argus*) were placed within an observation area of approximately 3 m² in the lagoon. Other *B. argus* were eviscerated, and the collected fishes were brought to the observation area. We noted into which sea cucumber the released fish entered. Then, all the holothurians in the area were collected to check whether they had contained fish before the experiment.

The goal of the next three experiments was to investigate the reaction of the holothurian to the penetration of fish, and the reaction of *C. boraborensis* and *C. homei* to their hosts' defence systems.

Experiment E

Five *B. argus* were placed in a 120-l aquarium (0.8 m×0.5 m×0.4 m). Their respiration rate (number of exhaling currents per minute) at rest was measured when fish were absent from the aquarium, and again when a fish was introduced into the aquarium. In order to evaluate the duration of expiration, the sea cucumbers were disturbed until emission of the Cuvierian tubules. During expirations, these tubules moved as a result of the water current produced by exhalation.

Experiment F

The responses of holothurians to different stimulations were tested in the field. A small wooden rod (diameter = 1 cm, length = 5 cm) was initially introduced into the cloaca of 46 *B. argus* specimens. The longest stimulation was about 5 min. Great care was taken to avoid water movements that could induce false responses and to prevent tissue injuries. If no response was elicited, these same specimens were then stimulated at the tentacles, the mouth, mid-way between the anus and mouth on the ventral surface of the body, and around the anus by hand with light pressure until the Cuvierian tubules were emitted. We noted which stimuli elicited expulsion of the Cuvierian tubules.

Experiment G

Small aquariums (1.2 m×0.6 m×0.2 m) were divided into three separate compartments (Fig. 3).

For each experiment, three sea cucumbers were placed in the centre (A) of the aquarium, while specimens of Carapidae [*C. homei* (0.4–5.9 g) + *C. boraborensis* (0.7–13.3 g)] were distributed in the external compartments (B and C) of the aquarium (Fig. 3). Different intact specimens of carapids (four *C. homei* and five *C. boraborensis*) were placed in compartment B. The mucus of five *C. homei* and five *C. boraborensis* specimens that were placed in compartment C had been removed 5 min before the experiment, using absorbing paper. Specimens of Apogonidae (one *Apogon fraenatus*, 5 g), Acanthuridae (eight *Acanthurus triostegus*, 1.1–2 g), Pomacentridae (two *Chrysiptera unimaculata*, 3.3 and 4.9 g; four *Chrysiptera glauca*, 0.6–1.7 g; three *Pomacentrus pavo*, 3.3–5.5 g and four *Dascyllus aruanus*, 2–6.9 g) were used as controls and were distributed in compartments B and C during the two sets of experiments. Strong pressure by hand was then applied to each of the three sea cucumbers until the Cuvierian tubules

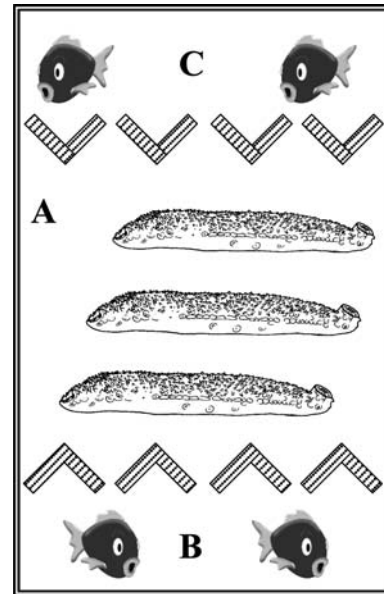


Fig. 3 Dorsal schematic illustration of the aquarium used for experiment IIC (A, B, C different sections)

were expelled. Because of the absence of current, it was assumed that the toxins spread out uniformly in the aquarium. The lapse of time that it took for the fish to die was then noted.

The relationship between the weight of the carapids and the time required for them to die was examined using Pearson's correlation coefficient.

Results

Part I: sampling

A total of 257 *Bohadschia argus* specimens were collected. Of these sea cucumbers, 84% contained the following fish species: *Carapus homei*, *C. boraborensis* and *Encheliophis gracilis*. A total of 85 *B. argus* sheltered 90 *C. homei* (39.3% of the infested sea cucumbers), 128 contained 136 *C. boraborensis* (59.2%) and 3 were occupied by 3 *E. gracilis* (1.3%). On the other hand, 81% of the 33 *Thelenota ananas* specimens contained 37 *C. boraborensis*, and 15% of the 45 *Culcita novaeguineae* specimens contained 7 *C. mourlani*. A total of 21 *Holothuria fuscogilva* and 18 *H. edulis* did not contain any Carapidae. Both specimens of *T. anax* contained 1 *C. boraborensis* each. In two cases, 1 *C. homei* and 1 *C. boraborensis* were found in the same sea cucumber (*B. argus*). The distributions of *C. boraborensis* and *C. homei* were similar to those of the sea cucumber hosts. These fishes were not present in the mouth of the bay, where the usual sea cucumber hosts (*B. argus*, *T. ananas*) were absent; only *H. fuscogilva* were found there. The distribution of Carapidae in the sampling area seems completely random.

Fourteen cases of infestation by male–female pairs of *C. boraborensis* were recorded. In addition, it was

possible for the first time to detect sexual dimorphism in *C. boraborensis*. Males were smaller than females, with a mean total length (TL) of 17.3 cm (± 2.2 , $n=27$), and none exceeded 20 cm (Fig. 4). Females had a mean TL of 21.7 cm (± 2.8 , $n=85$), with the largest specimen recorded at 31 cm. It was not possible to distinguish the sex of 18 specimens. Females were also characterised by a larger head and the absence of a distinct bulb at the posterior end of the swim bladder (Fig. 5). In general, males captured during this period appeared emaciated compared to females. The sex ratio for this population was three females to one male.

The sex ratio of *C. homei* was one female to one male, and they did not exhibit sexual dimorphism. The mean TL was 12.5 cm (± 2.6 , $n=19$) for males and 14.5 cm (± 1.0 , $n=20$) for females (Fig. 6). Pairs were not recorded, but six *C. homei* were recorded in the same sea cucumber. In this case, a male (15.5 cm TL) was in the presence of five females (14.1–15.0 cm TL), and these six specimens presented particularly well-developed gonads. This kind of multiple infestation has previously been observed (authors' personal observations). It is important to note that these multiple infestations were recorded in sea cucumbers that were not isolated in the bay, but rather were surrounded by other sea cucumbers inhabited or uninhabited by specimens of *Carapus* spp.

Part II: characterisations of the relationships between the fish species

Experiment A

Whatever the species, each specimen tested exhibited the same behaviour: the fish entered into the first sea cucumber with which they came into contact. All fish entered a host within a half-hour. Neither the presence nor the absence of another fish inside the holothurian host appeared to influence the choice (Table 1). Furthermore, the fish did not "test" several sea cucumbers, and specimens of different species simultaneously tried to find the opening of a single sea cucumber.

Experiment B

For both tests, two *C. boraborensis* and two *C. homei* were found dead in the tank between the 1st and 5th day; their caudal end had been eaten. During the same time period, one *C. boraborensis* and one *C. homei* were also found dead in the tank, but did not exhibit any apparent bite marks. Perhaps these fish did not survive the marking procedure. After 10 days of experimentation, two *C. homei* were not found, which indicated that they had been entirely eaten. At the end of the experiment, the occupation of the hosts was as follows: six sea cucumbers were occupied by the pairs of *C. homei*–*C. boraborensis* artificially formed at the beginning; five were occupied by only one fish, since some had been eaten; two were unoccupied by fish; and one sea cucumber sheltered a pair of *C. homei*–*C. boraborensis*, which had not been artificially formed at the beginning of the experiment.

Experiment C: Y-test

Odour resulting from the sea cucumber versus seawater A total of 27 *C. boraborensis* chose the branch containing water charged with the odour of sea cucumbers, 5 chose the other branch, and 14 did not leave the starting area. Eight *C. homei* chose the branch with odour-charged water, three went into the other branch, and four did not leave the starting area. With a χ^2 of 15.98, the assumption related to random fish distribution must be rejected: the choice of *C. boraborensis* appears to be guided by the water current charged with sea cucumber odour. On the other hand, $\chi^2=2.8$ for *C. homei* indicates that the assumption of random fish distribution must be accepted. However, the latter results could be biased because of the low number of trials. Thus, carapids do not seem to be influenced by conditioning of the water.

Influence of conspecific or another Carapidae Eight *C. homei* chose the branch where their conspecific was found, nine took the branch occupied by *C. boraborensis*,

Fig. 4 *Carapus boraborensis*. Total length (TL, cm) of males and females

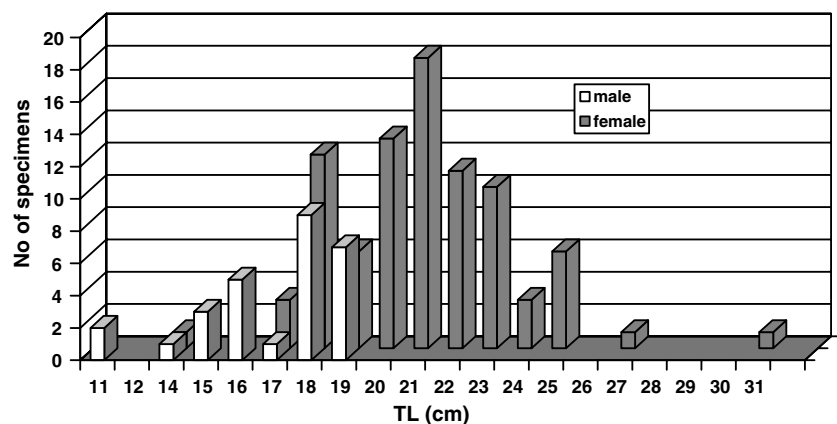


Fig. 5A, B *Carapus boraborensis*. Comparison of different morphological features between males (A) and females (B) (1 posterior end of the swim bladder; 2 dorsal view of the cranium; 3 lateral view of the cranium)

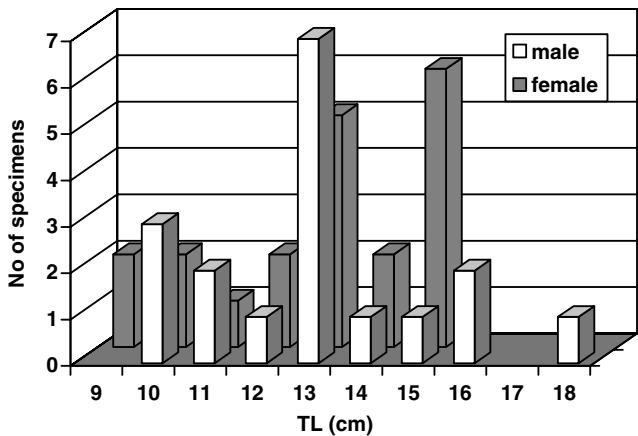
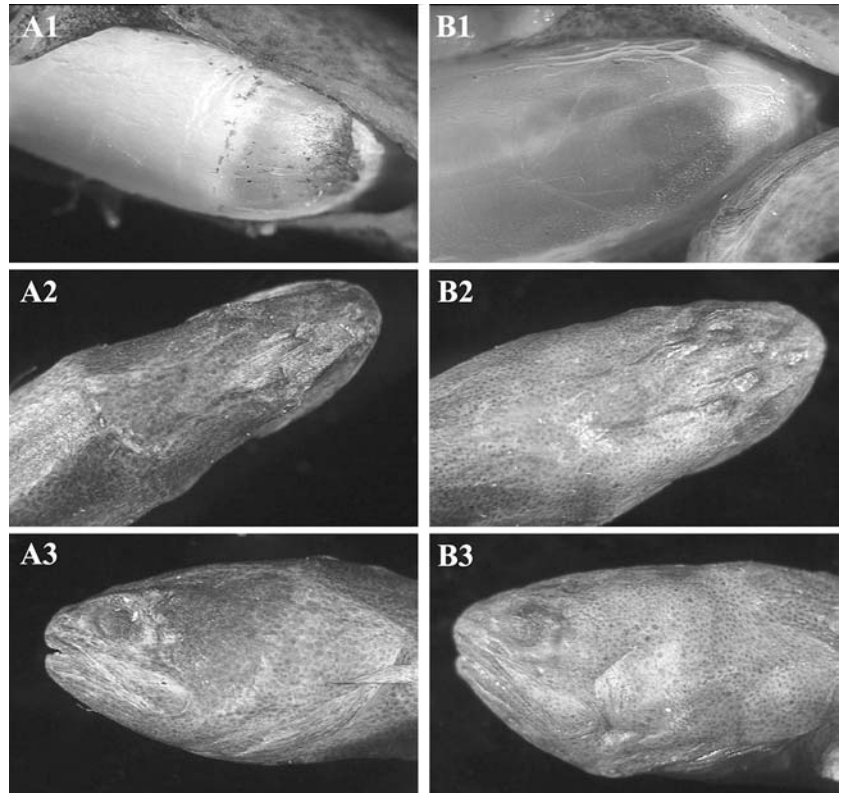


Fig. 6 *Carapus homei*. Total length (TL, cm) of males and females

and three did not leave the starting area ($\chi^2=2.8$). A total of 15 *C. boraborensis* chose the branch where their conspecific was found, 10 took the branch occupied by *C. homei*, and 17 did not leave the starting area ($\chi^2=1.85$). As the two χ^2 values are < 5.99 , the distribution of the two species does not appear to be influenced by the presence of conspecifics or carapids of the other species.

It is particularly interesting to note that during the experiments, carapids placed in the compartments of the Y branches (A and B, Fig. 2) tried to penetrate the provisioning pipe only when there was water flow. First, the fish attempted to enter the pipe, headfirst. The

Table 1 *Carapus* spp. Distribution of Carapidae fish in the sea cucumber (*Bohadschia argus*) during experiment IIA

Holothurian no.	Fish entering the holothurian	Infestation of the holothurian before the experiment
1	2 <i>C. boraborensis</i>	1 <i>C. boraborensis</i>
2	1 <i>C. boraborensis</i>	Empty
3	1 <i>C. homei</i>	1 <i>C. boraborensis</i>
4	3 <i>C. homei</i> +1 <i>C. boraborensis</i>	1 <i>C. boraborensis</i>
5	1 <i>C. homei</i> +2 <i>C. boraborensis</i>	1 <i>C. boraborensis</i>
6	1 <i>C. homei</i> +1 <i>C. boraborensis</i>	1 <i>C. homei</i>
7	1 <i>C. homei</i>	1 <i>C. homei</i>
8	2 <i>C. homei</i>	2 <i>C. homei</i>
9	2 <i>C. boraborensis</i>	1 <i>C. boraborensis</i>
10	None	1 <i>C. boraborensis</i>
11	None	Empty

attempts proved unsuccessful, because the entry to the pipe was blocked by a net. The fish then brought its caudal end forward, placed it against the grid and tried to enter the pipe, tail first.

Experiment D

The results of this field experiment were identical to those observed in the laboratory. Seven *C. boraborensis* and five *C. homei* specimens entered the first sea cucumber with which they came into contact. The fish did not show a preference between sea cucumbers, and the presence or absence of another fish inside the sea cucumbers did not influence behaviour.

Part III: sea cucumber and fish defences

Experiment E

During the observations, the sea cucumber expired on average every 8 s (7.89 ± 1.5 , $n=49$), with expirations lasting approximately 2 s. This rhythm was interrupted by longer and stronger expirations of approximately 10 s. Most frequently, the latter appeared after five short breathings. This frequency was disturbed when the fish presented itself in front of the sea cucumber's cloaca and touched it. In this case, the sphincters contracted and the sea cucumber began an "apnea". When the sea cucumber relaxed its sphincters to respire, the fish felt the water current on its head and brought its caudal portion forward, using its body as a guide. As the tail entered the cloaca, it obstructed it, and the sea cucumber widened the cloaca opening in order to respire. The fish at this point could then penetrate more deeply inside. If the fish passed in front of the cloaca and felt the exhaling current produced by the sea cucumber, it penetrated headfirst, before the host contracted its sphincter.

Experiment F

At sea, 2 of the 46 sea cucumbers released their Cuvierian tubules when a rod was inserted into the cloaca. Forty-two sea cucumbers expelled their Cuvierian tubules following tactile simulation, and two sea cucumbers did not exhibit any reaction. With each Cuvierian tubule expulsion, the sea cucumber bent laterally and brought its cloaca forward, in order to place the Cuvierian tubules against its mouth tentacles.

Experiment G

At the time of the Cuvierian tubules' expulsion, all reef fish other than carapids died within 3–6 min. Death was preceded by frenzied body movements accompanied by a rapid acceleration in respiration. The fish then put themselves on their flanks, and only opercular movements occurred until death. There was no correlation between the time preceding death and body weight (0.6–6.9 g).

The first reaction of carapids to the Cuvierian tubules' expulsion could be described as a "sneeze". Later, they gradually positioned themselves on their backs and exhibited brief swimming movements before dying. However, all the carapids were clearly distinguished from the other reef fish tested by their greater resistance to the toxins of the Cuvierian tubules. The time required to die varied from 20 min to >160 min (Fig. 7). The manual removal of mucus did not have a significant effect on this delay.

If all Carapidae are analysed as one group, the time required to die is significantly correlated with weight ($r^2=0.806$, $P<0.0001$, $n=19$; Fig. 7). This correlation is also significant if *C. boraborensis* specimens are taken

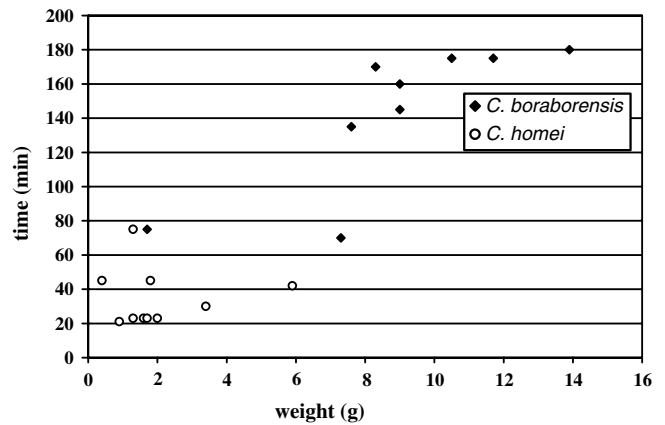


Fig. 7 Time (min) required to die in *Carapus boraborensis* (filled points) and *Carapus homei* (open points) as a function of their weight

separately ($r^2=0.65$, $P<0.01$, $n=9$), but this is not the case for *C. homei* ($n=10$).

Discussion

Two species cannot occupy the same ecological niche unless they enter into competition. Generally speaking, an ecological niche is defined by habitat as well as by the functional relationships (e.g. food, competitors, predators, etc.) between an organism and its ecosystem. *Carapus homei* and *C. boraborensis* live in the same sea cucumber species, in the same waters and are both commensal. The only differences actually detected concern reef colonisation, during which their larvae are not in the same developmental state (Parmentier et al. 2002). Other behavioural differences might also exist, since their otolith microstructures show different deposition patterns. The different results of the present field and laboratory studies highlight some behavioural features and help to determine ecological niche characteristics. These experiments also provide a better understanding of the relationships between hosts and Carapidae and of their respective defence systems.

Part I: contribution of sampling

This study revealed for the first time the existence of sexual dimorphism in *C. boraborensis* and the possibility of a male and female coexisting in the same host. The presence of sexual pairs in sea cucumbers has also been observed in the *Encheliophis* parasites (Murdy and Cowan 1980; Trott 1981). A multi-infestation of more than two individuals has been observed, however, in other species like *C. homei*, *C. mourlani* and *C. bermudensis* (Aronson and Mosher 1951; Trott and Trott 1972; Meyer-Rochow 1977; Smith et al. 1981).

The sex ratio difference between *C. homei* (1:1) and *C. boraborensis* (3:1) could be related to their repro-

ductive behaviour. During this study, which was conducted in June, *C. boraborensis* males appeared emaciated, which was not the case for females and all other heterospecifics. This emaciation could have resulted from the search for several sexual partners in various hosts. This hypothesis was reinforced by the presence of eggs found in an aquarium where a female had been placed with a male. In *C. bermudensis*, the multi-infestation could have coincided with the reproduction period (Smith et al. 1981). In *C. homei*, the multi-infestation was observed at a site where the host was surrounded by empty sea cucumbers or ones occupied by only one *Carapus*. The regrouping of six specimens with well-developed gonads within a lone sea cucumber could not be a coincidence, and may testify to a different reproductive behaviour.

The early life history of carapids is not well documented. The ellipsoidal eggs are emitted in a mucilaginous raft, which confers buoyancy (Emery 1880; Branch 1969; Tyler et al. 1992). Vexillifer larvae of *Carapus* are usually found outside the lagoon (Ambrose 1996; Olney et al. 2000). These data indicate that hatching is not present in the sea cucumber, but that the eggs are emitted into the lagoon and carried by the currents to the pelagic ocean before hatching, as is the case for the majority of coral fish (Leis 1991; McCormick and Makey 1997; Bonhomme and Planes 2000; Leis and McCormick 2002). The Carapini reproductive behaviour has never been described in detail, but different elements suggest that coupling occurs inside the sea cucumber. Male and female individuals are found in the same host, reproduction in the respiratory tree would greatly favour the gametes meeting, and the adults and their eggs are temporarily sheltered from predators during fecundation. This reproduction under the protection of the host has also been observed in clownfish (Allen 1972; Fautin 1991; Averdlund and Nielsen 1996; Arvedlund et al. 2000a). In the latter, it would also allow imprinting of the eggs with the host odour, which could constitute an advantage at the time of colonisation, with different post-larval clownfish identifying their hosts on the basis of chemical stimuli (Averdlund and Nielsen 1996). However, an innate behaviour cannot be excluded (Fautin 1991; Miyagawa 1989; Arvedlund et al. 1999, 2000b). The tennis larvae, which do not have any previous contact with a host, are also able to recognise it at the time of colonisation.

Part II: characterisations of the relationships between fish species

When a clownfish occupies a sea anemone, it can prevent the intrusion of other heterospecifics and, in certain cases, conspecifics (Fautin 1991). Past studies of *C. boraborensis*, *C. homei* and *E. gracilis* revealed that these species can live in the same sea cucumber species, but the literature does not report any infestation case in which heterospecifics have been found in the same host (Smith

1964; Trott 1970; Trott and Trott 1972; Van Den Spiegel and Jangoux 1989). The present study has revealed for the first time multispecific infestation; the latter remains very weak (> 1%), but is nevertheless possible. The four laboratory experiments and the field study revealed that the fish do not appear to be able to determine if a potential host is already occupied or not. The fish penetrates the first host with which contact is established. These results confirm sound emission data (Parmentier et al. 2003). In aquaria, fish emit sounds only when they enter an already occupied host. No sound was recorded during the fish's approach of the sea cucumber or during the search for the cloaca (Parmentier et al. 2003). The apparent chemical and sonic discretion of fish during host approach could be related to diet. Since these species are capable of eating one another, the specimens entering and those already resident may seek an advantage by not making their presence known. Nevertheless, experiment B also revealed that the meeting of two species does not automatically involve a feeding behaviour.

Part III: defence of the sea cucumber and defence of the fish

In *C. bermudensis*, *C. acus* and *E. vermicularis*, laboratory experiments indicated that the host localisation is primarily due to chemical stimuli. Sight could also play a role, but vision does not appear as important as chemical stimuli. Aronson and Mosher (1951) demonstrated that experimentally blinded *C. bermudensis* are able to locate their host, while Meyer-Rochow and Tiang (1978) noted that the eyes of *C. mourlani* have characteristics similar to those of cavernicolous or deep-sea organisms. The present study indicates the capacity of *C. boraborensis* to locate its host thanks to chemical stimuli. According to Trott (1970), *C. homei* does not appear to be able, at least under laboratory conditions, to locate its host on the basis of chemical stimuli. However, the χ^2 in this study was calculated with negative (wrong branch of the Y) and null trials (fishes do not move). When the fish moved, it entered the odour-charged branch in 72% of the cases. Additional experiments should be carried out with this species. In addition, specimens of the two fish species reacted to the physical stimulus of a water current being emitted by a pipe, a behaviour also observed in *C. acus* (Arnold 1957).

When the host is localised, carapids are generally in an almost vertical position with their head at the bottom. They then search the sea cucumber by touching it with their nose. This behaviour and the fact that they have to search the host several times before locating the cloaca indicate that the fish does not instinctively know the position of the sea cucumber's cloaca. The latter can only be localised when a respiratory water current is emitted during fish passage. Two kinds of penetration behaviour, headfirst or tail first, have previously been described (Arnold 1953; Kloss and Pfeiffer 2000). This

duality appears to be related to the sea cucumber's behaviour. One, the expiratory current of the sea cucumber is rather strong, i.e. the anus is sufficiently dilated, and the fish physically has the space to pass headfirst. Two, the presence of the fish is detected and the sea cucumber closes its cloaca and begins an "apnea". A fish that feels the water current but cannot penetrate directly into the sea cucumber remains in front of the cloaca. The sea cucumber must, however, finally stop its apnea and ends up exhaling. As soon as the fish feels the water current, it brings its caudal end forwards and enters it into the cloaca. The introduction of the caudal end obstructs the opening, which stimulates the sea cucumber to dilate its sphincter more in order to breathe, allowing the fish to enter deeply into the host. This explains the difference in penetration behaviour between adults and juveniles. The adults, which are larger, enter in 80% of the cases tail first, while this proportion is completely reversed for juveniles (Gustato et al. 1979), because their smaller cranium does not require as broad an opening to pass headfirst.

The Cuvierian tubules consist of several hundred tubules the proximal end of which is attached to the base of the left respiratory tree and the distal end of which hangs freely in the body cavity (Van Den Spiegel et al. 2000). When it is irritated, the animal expels its tubes from its body and, thus, releases their toxic contents (Bakus 1968; Habermelch and Krebs 1990). Tactile stimulations applied to the tegument of various sea cucumber species show that there is an antero-posterior gradient of sensitivity, which could be related to the greater vulnerability of the oral tentacles compared to the remainder of the body (Bakus 1973; Lawrence 1987; Hamel and Mercier 2000). This expulsion behaviour was also observed when the sea cucumber was disturbed by different predators that could give up their potential prey (Hamel and Mercier 2000). In *B. argus*, tactile stimulation automatically involved movement of the body in order to place the Cuvierian tubules against the mouth. On the other hand, stimulation by penetration of an object into the cloaca was followed by expulsion of the tube in 6% of the cases. Consequently, the absence of a discharge of the Cuvierian tubules when carapids penetrate the cloaca (Trott 1981) may not be related to a particular feature or fish behaviour, as outlined in Van Den Spiegel and Jangoux (1989). The crab species that is also capable of penetrating the respiratory tree of the sea cucumber (Wells and Wells 1961; Crosnier 1962; Trott and Garth 1970; Ng and Jeng 1999) does not provoke expulsion of the Cuvierian tubules either (authors' personal observations). Curiously, this defence system seems to have developed to dissuade possible predators of the sea cucumber's external tissue, but appears to be ineffective in discouraging those species that penetrate the respiratory trees.

Sea anemones release toxic compounds, which, in sufficient concentrations, can deteriorate the epithelial cells of clownfish gills and can result in their death (Mebs 1994; Mebs et al. 1996). The mucus of these fish appear to constitute an adaptation to prevent the nematocysts'

entry into the epidermic tissue and/or to prevent their discharge (Lubbock 1980; Fautin 1991; Elliott and Mariscal 1996). The defence system seems to be different in carapids, which do not suffer mechanical attacks from the Cuvierian tubules. The latter produce a sticky and toxic substance, but ichthyotoxins are also produced at the level of the sea cucumber tegument (Van Den Spiegel and Jangoux 1987; Habermelch and Krebs 1990; Bryan et al. 1997). These toxins are saponins derived from triterpenoids with strong haemolytic, neurotoxic and membranolytic action (Habermelch and Krebs 1990; Kalinin et al. 1996). In a natural environment, these toxins mainly have a dissuasive effect (Bryan et al. 1997; Hamel and Mercier 2000), but they are lethal in aquaria (Nigrelli 1952; Bakus 1968). Trott (1970) observed an increase in mucus production when extracts of Cuvierian tubules were presented to *E. vermicularis*, but there was no significant difference in the time of survival of *C. boraborensis* and *C. homei* in the presence or absence of mucus in the present study. A second method of toxin entry is at the gills, where it can cause the rupture of capillaries (Nigrelli 1952; Verbist 1993). Because of the greater resistance of *C. homei* and *C. boraborensis* to toxins, it might be interesting to examine their gills. However, the mucus also allows carapids to move easily in the Cuvierian tubules without being hindered by the sticky substance the tubules emit (Trott 1970). These fish are also devoid of features that could present grips: there are no scales, the opercle spines are prolonged by soft tissues and the fin rays are flexible and not thorny.

As in certain clownfish, the protection system of carapids could be twofold: a non-dependent system, which is represented by the absence of discharge of the Cuvierian tubules when the fish enters into the host and the possible resistance of the gills to toxins. The latter still remains to be shown.

Not all sea cucumber species can be occupied by carapids; two hypotheses can be postulated here to explain this.

1. The initial relation of carapids to their hosts seems to be guided by a search for shelter. This behaviour may only shift the problem, since the fish "adopts" the predators of its host. All the known hosts of Carapini species (*C. boraborensis*, *C. homei*, *C. bermudensis*, *C. acus* and *E. gracilis*) are sea cucumbers with Cuvierian tubules. Is it possible to see here a result of natural selection, in which the choice of a host equipped with a defence system could minimise the risk of predation?
2. Carapini, like the majority of fishes, breathe through gills. From a mechanical point of view, breathing consists of the mandible lowering and the spacing of the suspensoria and the opercles. This succession of movements involves an increase in volume of the oral cavity, the pharyngeal cavity and the opercular cavity, creating a water current irrigating the gills. These movements are possible only if the different parts can move away from the body. In sea cucumbers,

Carapini can be localised in the body cavity, but are mainly found in the respiratory trees (Branch 1969; Trott 1970; Gustato 1976; Van Den Spiegel and Jangoux 1989; authors' personal observations), where the fish can benefit from the water flow during respiration of the sea cucumber. The respiratory tree diameter of certain sea cucumber species is too small to allow Carapini to live there. In certain species like *Holothuria fuscogilva*, the presence of anal teeth at the level of the sea cucumber cloaca could also be a limiting factor. These triangular teeth could seriously hurt fish trying to enter into the host.

Acknowledgements We thank Dr. R. Galzin, Dr. Y. Chancerelle, J. Algret and J. Million (CRIOBE, Moorea, French Polynesia), Dr. V. Dufour and C. Brié (Tropical Fish Rangizoa) for assisting us in obtaining live carapids. This work was supported by grant no. 2.4560.96 from the "Fonds National de la Recherche Scientifique" of Belgium.

References

- Allen GR (1972) The anemonefishes: their classification and biology. TFH Publications, Neptune City, N.J., USA
- Ambrose DA (1996) Carapidae: pearlfishes. In: Moser HG (ed) The early stages of fishes in the California current region. Calcofi atlas no. 33. Allen, Lawrence, Kan., pp 532–533
- Arnold DC (1953) Observation on *Carapus acus* (Brünnich) (Jugulares, Carapidae). *Pubbl Staz Zool Napoli* 24:152–166
- Arnold DC (1956) A systematic revision of the fishes of the teleost family Carapidae (Perciformes, Blennioidea), with description of two new species. *Bull Br Mus (Nat Hist) Zool* 4:247–307
- Arnold DC (1957) Further studies on the behaviour of the fish *Carapus acus* (Brünnich). *Pubbl Staz Zool Napoli* 30:263–268
- Aronson LH, Mosher C (1951) Observations on the behaviour and ecology of the West Indian pearlfish. *Anat Rec* 111:489
- Averdlund M, Nielsen LE (1996) Do the anemonefish *Amphiprion ocellaris* (Pisces: Pomacentridae) imprint themselves to their host anemone *Heteractis magnifica* (Anthozoa: Actinidae)? *Ethology* 102:197–211
- Averdlund M, McCormick M, Fautin DG, Bildsøe M (1999) The anemonefish *Amphiprion melanopus* (Bleeker) (Pisces: Pomacentridae): a study of host detection and possible imprinting. *Mar Ecol Prog Ser* 188:207–218
- Averdlund M, Bundgaard I, Nielsen LE (2000a) Host imprinting in anemonefishes (Pisces: Pomacentridae): does it dictate spawning site preferences? *Environ Biol Fishes* 58:203–213
- Averdlund M, Larsen K, Winsor H (2000b) The embryonic development of the olfactory system in *Amphiprion melanopus* (Perciformes: Pomacentridae) related to the host imprinting hypothesis. *J Mar Biol Assoc UK* 80:1103–1110
- Bakus GJ (1968) Defensive mechanisms and ecology of some tropical holothurians. *Mar Biol* 2:23–32
- Bakus GJ (1973) The biology and ecology of tropical holothurians. In: Jones OA, Endean R (eds) *Biology and geology of the coral reefs*, vol 2. Academic, New York, pp 325–367
- Bauchot MLB (1992) Poissons téléostéens. In: Tillier S (ed) *Encyclopédie du monde animal*. Bordas, Paris, pp 367–368
- Bonhomme F, Planes S (2000) Some evolutionary arguments about what maintains the pelagic interval in reef fishes. *Environ Biol Fishes* 59:365–383
- Branch JB (1969) Observations on the ecology and behaviour of Guam pearlfishes (Carapidae). *Micronesica* 24:274
- Bryan PJ, McClintock JB, Hopkins TS (1997) Structural and chemical defences of echinoderms from the northern Gulf of Mexico. *J Exp Mar Biol Ecol* 210:173–186
- Castro-Aguirre JL, Garcia-Dominguez F, Balart EF (1996) Nuevos hospederos y datos morfológicos de *Encheliophis dubius* (Ophidiiformes: Carapidae) en el Golfo de California, México. *Rev Biol Trop* 44:753–756
- Cheney DP (1973) Pearlfish (Carapidae) in *Acanthaster planci* (L.). *Micronesica* 9:159
- Crosnier A (1962) Crustacés décapodes Portunidae. *Faune de Madagascar* 16:1–154
- Elliott JK, Mariscal RN (1996) Ontogenetic and interspecific variation in the protection of anemonefishes from sea anemones. *J Exp Mar Biol Ecol* 208:57–72
- Emery DC (1880) Fierasfer. Studi intorno alla sistematica, l'anatomia e la biologia delle specie mediterranee di questo di genere. *Atti Accad Naz Lincei Cl Sci Fis Mat Nat Mem* 7:167–316
- Fautin DG (1991) The anemonefish symbiosis: what is known and what is not. *Symbiosis* 10:23–46
- Gustato G (1976) Osservazioni sulla biologica e sul comportamento di *Carapus acus* (Ophioidei, Percomorphi). *Boll Soc Nat Napoli* 85:505–535
- Gustato G, Villari A, Villani G (1979) Ulteriori dati sul comportamento di *Carapus acus* (Gadiformes, Ophidiodei). *Boll Soc Nat Napoli* 88:535–547
- Habermelth GG, Krebs HC (1990) Toxins of echinoderms. In: Attatur-Rahman (ed) *Studies in natural products chemistry*, vol 7. Elsevier Science, Amsterdam, pp 265–316
- Hamel JF, Mercier A (2000) Cuvierian tubules in tropical holothurians: usefulness and efficiency as a defence mechanism. *Mar Freshw Behav Physiol* 33:115–139
- Kalinin VI, Prokofieva NG, Likhatskaya GN, Schentsova EB, Agafonova IG, Avilov SA, Drozdova OA (1996) Hemolytic activities of triterpene glycosides from the holothurian order dendrochirotida: some trends in the evolution of this group of toxins. *Toxicon* 34:475–483
- Kloss K, Pfeiffer W (2000) Zur Biologie des "Eingeweidefisches" *C. acus* (Brünnich, 1768) (Carapidae, Teleostei), mit Hinweisen auf eine nicht-parasitische Ernährung. *Rev Suisse Zool* 107:335–349
- Lawrence JM (1987) A functional biology of echinoderms. The Johns Hopkins University Press, Baltimore, Md., USA
- Leis JM (1991) The pelagic stages of reef fishes: the larval biology of coral reef fishes. In: Sale PF (ed) *The ecology of fishes on coral reefs*. Academic, San Diego, pp 183–227
- Leis JM, McCormick MI (2002) The biology, behaviour, and ecology of the pelagic, larval stage of coral reef fishes. In: Sale PF (ed) *Coral reef fishes: new insights into their ecology*. Academic, San Diego
- Lubbock R (1980) Why are clownfishes not stung by sea anemones? *Proc R Soc Lond B* 207:35–61
- Mader SS (1987) *Biologie: évolution, diversité et environnement*. Goulet, Ottawa, Canada
- Markle DF, Olney JE (1990) Systematics of the pearlfish (Pisces: Carapidae). *Bull Mar Sci* 47:269–410
- McCormick MI, Makey LJ (1997) Post-settlement transition in coral reef fishes: overlooked complexity in niche shifts. *Mar Ecol Prog Ser* 153:247–257
- Mebs D (1994) Anemonefish symbiosis: vulnerability and resistance of fish to the toxin of the sea anemone. *Toxicon* 32:1059–1068
- Mebs D, Giese C, Werdling B (1996) The gills of fish and crustaceans: vulnerability and resistance to cytotoxic toxins. *Toxicon* 34:333
- Meyer-Rochow VB (1977) Comparison between 15 *Carapus mourlani* in a single holothurian and 19 *Carapus mourlani* from starfish. *Copeia* 1977:582–585
- Meyer-Rochow VB (1979) Stomach and gut content of *Carapus mourlani* from starfish and a holothurian. *Ann Zool Fenn* 16:287–289
- Meyer-Rochow VB, Tiang MK (1978) Visual behavior, eye and retina of the parasitic fish *Carapus mourlani*. *Biol Bull (Woods Hole)* 155:576–585
- Miyagawa K (1989) Experimental analysis of the symbiosis between anemonefishes and sea anemones. *Ethology* 80:19–46

- Murdy EO, Cowan ME (1980) Observation on the behaviour and symbiotic relationship of the pearlfish *Encheliophis vermicularis* (Osteichthys: Carapidae). *Kalikasan* 9:309–312
- Ng PKL, Ming-Shiou Jeng (1999) The brachyuran crabs (Crustacea: Decapoda: Eumedonidae and Portunidae) symbiotic with echinoderms in Taiwan. *Zool Stud* 38:268–274
- Nigrelli RR (1952) The effects of holothurin on fish, and mice with sarcoma 180. *Zoologica* 37:89–90
- Olney JE, Leis JM, Rennis DS (2000) Carapidae. In : Leis JM, Carson-Ewart BM (eds) *The larvae of Indo-Pacific coastal fishes*. Brill, Leiden, pp 104–107
- Parmentier E, Vandewalle P (2003) Morphological adaptations of pearlfish (Carapidae) to their various habitats. In: Val AL, Kapoor BG (eds) *Fish adaptations*. Science Publishers, Enfield, USA, pp 261–276
- Parmentier E, Castro-Aguirre JL, Vandewalle P (2000) Morphological comparison of the buccal apparatus in two bivalve commensal Teleostei: *Encheliophis dubius* and *Onuxodon fowleri* (Carapidae, Ophidiiformes). *Zoomorphology* 120:29–37
- Parmentier E, Lo-Yat A, Vandewalle P (2002) Identification of four French *Polynesia tenuis* carapini (Carapidae: Teleostei). *Mar Biol* 140:633–638
- Parmentier E, Vandewalle P, Lagardère JP (2003) Sound producing mechanisms and recordings in three Carapidae species. *J Comp Physiol A* 189:283–292
- Scherrer B (1984) *Biostatistiques*. Gaëtan Morin, Québec, Canada
- Shen SC, Yeh HS (1987) Study on pearlfishes (Ophidiiformes: Carapidae) of Taiwan. *J Taiwan Mus* 40:45–56
- Smith CL (1964) Some pearlfishes from Guam, with notes on their ecology. *Pac Sci* 18:34–40
- Smith CL, Tyler JC, Feinberg MN (1981) Population ecology and biology of the pearlfish (*Carapus bermudensis*) in the lagoon at Bimini, Bahamas. *Bull Mar Sci* 3:876–902
- Trott LB (1970) Contribution of the biology of carapid fishes (Paracanthopterygian: Gadiformes). *Univ Calif Publ Zool* 89:1–41
- Trott LB (1981) A general review of the pearlfishes (Pisces, Carapidae). *Bull Mar Sci* 31:623–629
- Trott LB, Garth JS (1970) *Lissocarcinus orbicularis* Dana (Portunidae, Caphyrinidae), commensal with *Holothuria argus* Jaeger—a new host record; cohabitation with the pearlfish *Carapus homei* (Richardson). *Crustaceana* 19: 30–321
- Trott LB, Trott EB (1972) Pearlfishes (Carapidae: Gadiforme) collected from Puerto Galera, Minobra, Philippines. *Copeia* 1972:839–843
- Tyler JC, Robins CR, Smith CL, Gilmore RG (1992) Deepwater populations of the western Atlantic pearlfish *Carapus bermudensis* (Ophidiiformes: Carapidae). *Bull Mar Sci* 51:218–223
- Van Den Spiegel D, Jangoux M (1987) Cuvierian tubules of the holothuroid *Holothuria forskali* (Echinodermata): a morpho-functional study. *Mar Biol* 96:263–275
- Van Den Spiegel D, Jangoux M (1989) La symbiose entre poissons Carapidae et Holoturies autour de l'île de Laing (Mer de Bismarck, Papouasie Nouvelle Guinée). *Indo-Mal Zool* 6:223–228
- Van Den Spiegel D, Jangoux M, Flamang P (2000) Maintaining the line of defense: regeneration of Cuvierian tubules in the sea cucumber *Holothuria forskali* (Echinodermata, Holothuroidea). *Biol Bull (Woods Hole)* 198:34–49
- Van Meter VB, Ache BW (1974) Host location by the pearlfish *Carapus bermudensis*. *Mar Biol* 26:379–386
- Verbist JE (1993) Pharmacological effects of compounds from echinoderms. In: Jangoux M, Lawrence JM (eds) *Echinoderm studies*. Balkema, Rotterdam, pp 111–186
- Weber M (1913) Die fische der Siboga-Expedition. *Siboga-Exped* 32:1–710
- Wells HW, Wells M (1961) Observations on *Pinnaxodes floridensis*: a new species of pinnotherid crustacean commensal in holothurians. *Bull Mar Sci* 11:267–279