

# Optimal growth and feeding behaviour of the valuable bait *Halla parthenopeia* (Polychaeta: Oeonidae) in small-scale rearing systems

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## ABSTRACT

Commercial farming of polychaetes used as baits in recreational fishing and feed supplements for aquaculture has been regrettably limited to a few species. The rearing conditions and diet that optimize the survival and growth remain largely unknown for most species. This study aimed at improving the current knowledge on the rearing of the large bivalve-feeder polychaete *Halla parthenopeia*, one of the most appreciated and expensive baits by Mediterranean fishermen. We set up a laboratory rearing system for *H. parthenopeia* to address several management procedures and solutions aimed at improving the rearing method that has been employed so far. We also provide a detailed reconstruction of feeding behaviour and prey preferences which were unknown for the Mediterranean populations. Finally, we assessed growth when fed on commercial, intact clams or artificially opened clams. The latter fed has been never tested in previous studies on *Halla* species and simulates commercial waste clams, a potential low-cost resource for polychaete maintenance. The setting up of a grow-out system based on single worms maintained in small PET containers on the bottom of recirculating aquaria permitted the non-stressful manipulation of *H. parthenopeia* and an easy management of the aquarium system. We observed that polychaetes consumed both *C. gallina* and *R. philippinarum*, showing a nocturnal foraging behaviour consisting in three phases: searching, handling, and feeding. The duration of handling + feeding period (1–4 h) depended on bivalve size. Offering intact *R. philippinarum* clam, a significant positive correlation between the predator and prey size occurred *i.e.* the smaller the predator, the smaller the prey-size selected, probably because less time and energy were required for handling. The same behaviour was observed also for the congeneric *Halla okudai* and in *H. parthenopeia* from the Suez Canal. Polychaetes preferred feeding on open clams than intact clams, independently from clam and worm size. An increase in the daily predation rate (up to 300%) and growth increment (up to 250%) was found for *H. parthenopeia* feeding on open clam, maybe for the lack of energetic cost for prey handling. These results suggest that an indoor small-scale production of *H. parthenopeia* fed with waste clams could meet the demand of the angling community and support further biotechnological applications of this polychaete.

## 1. Introduction

Polychaetes are among the dominant components of marine benthic communities, playing critical roles in interspecific interactions (Rodrigo and Costa, 2019; Capa and Hutchings, 2021; Righi et al., 2022). Large carnivorous polychaetes consuming benthic invertebrates, such as amphipods, isopods, other polychaetes, ophiuroids and bivalves, can strongly modify population dynamics, distribution and diversity of prey (Jumars and Dorgan, 2015). Besides, laboratory feeding bioassays and field observations have shown that they are among the main prey of

several invertebrate and vertebrate benthic consumers (e.g., Kicklighter and Hay, 2006; Jumars and Dorgan, 2015). However, the vast majority of the 13,000 identified species are quite unknown to non-specialists, with the exceptions of those that serve as baits for angling (e.g., “bloodworms” and “ragworms”), are prized by tropical aquarists for their colourful crowns of tentacles (e.g., “Christmas tree worms”), or which can cause human injury such as fireworms. Therefore, it is not surprising that the economic value and biotechnological potential of marine polychaetes have been still largely unexplored (Cole et al., 2018; Rodrigo and Costa, 2019; Jerónimo et al., 2021; Righi et al., 2022).

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Only a few polychaete species have achieved great commercial value as baits in recreational fishing and feed supplements for cultured fish and crustaceans. (e.g., Olive, 1999; Nesto et al., 2012; Watson et al., 2017; Cole et al., 2018; Lim et al., 2021). In Europe, the market demand of polychaetes as bait is too high to be met by local supply, so most of the worms sold are imported from Eastern Asia or North America (Sá et al., 2017). Commercial farming of native species is considered an attractive solution to avoid biosecurity problems associated with live exports, reduce direct and indirect impacts of harvesting, promote the development of new aquaculture products, and provide individuals for the restocking of populations severely depleted by overexploitation (Gambi et al., 1994; Watson et al., 2017; Nesto et al., 2018; Pombo et al., 2020; Jerónimo et al., 2021). However, the culture of native polychaetes must be competitive in terms of price, quality, product diversity and environmental sustainability when compared to the available wild sources or imported species (Olive, 1999; Pombo et al., 2020).

The tube-dwelling oeonid *Halla parthenopeia* (Fig. 1) appears to be a promising candidate for polychaete aquaculture (Osman et al., 2010a, 2010b). It is a large infaunal worm (up to 1 m long, 30 g weight) that lives buried within coastal muddy-sandy sediments in coastal temperate and sub-tropical marine habitats, consuming bivalves (Osman et al., 2010a, 2010b; Iori et al., 2014; Mikac, 2015). In the western Mediterranean, *H. parthenopeia* is marketed as “Ver de chalu” (France), “Llobarrero” (Spain) or “Dragone” (Italy). It is among the most appreciated and expensive baits: its retail price is around to 350–500 € per kg, but the market price of large sized specimens (20–30 g) can be estimated 12–18 € each or even more (Baeta et al., 2019; Simonini pers. obs.). Fishery of *H. parthenopeia* exploits field populations, giving rise to a thriving collecting business. However, wild catch poses some drawbacks: the harvest shows strong seasonal oscillations, giving the supply of bait discontinuous throughout the year. Moreover, both direct and indirect impacts on marine and coastal ecosystems are caused (Baeta et al., 2019).

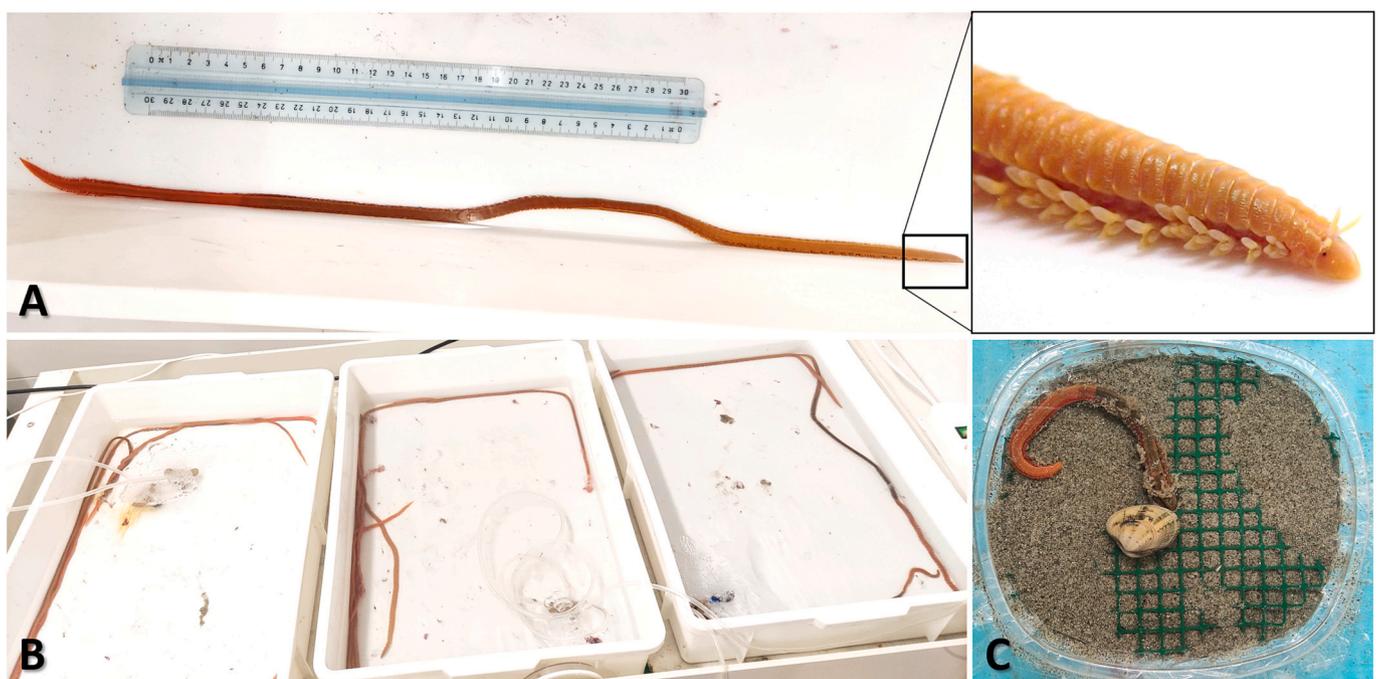
No successful case of practical aquaculture production of these worms has been available to date to our knowledge. Yet, study of the feeding behaviour and rearing trials in laboratory conditions were performed on two species belonging to the genus *Halla*: *H. okudai* from

Japan (Saito, 1994; Saito and Imabayashi, 1997; Saito et al., 1999; Saito et al., 2000; Saito et al., 2004; Imabayashi et al., 1996) and *H. parthenopeia* from Egypt (Osman et al., 2010a, 2010b).

*H. okudai* is a selective consumer, searching and choosing the most suitable and profitable prey. In Hiroshima Bay, the worm can prey on different bivalves, but its “health” (assessed through a condition factor) increases with increasing the density of the venerid clam *Ruditapes philippinarum* (Saito et al., 1999). This clam was also the preferred prey in most laboratory feeding trials. They showed that worm prey preferences depend on the bivalve species offered and the relative size of prey and predator: due to the high cost of prey handling, small worms tended to consume small-sized bivalves and vice versa (Saito et al., 1999, 2000, 2004). The feeding behaviour of *H. okudai* could be considered one of the most complex that has been observed among polychaetes so far. This worm displays nocturnal feeding habits including three main phases (i.e., searching, handling, and feeding). The worm seems to perceive the presence of the bivalve probably due to metabolites emitted by siphons and moves toward it dwelling in the sediment. Despite their burrowing behaviour, clams cannot escape predation: once in contact with the prey, the worm covers it with a paralytic mucus until the bivalve shell slightly opens. Feeding phase begins when *H. okudai* enters the bivalve to eat the soft tissues and stops when the worm leaves the empty shell returning to its burrow (Saito, 1994; Imabayashi et al., 1996; Saito et al., 2004).

A small-scale attempt to maintain and rear *H. parthenopeia* was performed with specimens collected from the Ismaila Lake (Suez Canal, Eastern Mediterranean) (Osman et al., 2010a, 2010b). It was evidenced that it can consume different venerid clams originally collected from the lake bottom, showing a feeding behaviour and preferences which resembles those of *H. okudai*. *H. parthenopeia* exhibits preferences for certain clams like *Paphia aurea* and *Ruditapes decussatus* and can grow under laboratory conditions (Osman et al., 2010a). In both *Halla* species, the diet based on live clams forces the worms to allocate a huge amount of time and resources to prey manipulation at the expense of growth (Saito et al., 2004).

As regard with the western Mediterranean populations of *H. parthenopeia*, the little information available to date comes from the



**Fig. 1.** *Halla parthenopeia* acclimatation and maintenance. A) Single whole specimen (24.7 g weigh, about 60 cm length) with a detailed view of its anterior body part (inlet). B) Several specimens with different size housed in HDPE flat tanks (60 × 40 cm) before their allocation in the experimental tanks. C) Set up of PET containers covered with a HDPE net below the sediment surface, housing one worm specimen and a clam.

Authors' research focused on the venoms involved in defence and predation (Iori et al., 2014; Simonini et al., 2019). *H. parthenopeia* produces a purple mucus containing hallachrome, an unusual 1–2 anthraquinone that shows strong toxicity against bacteria, protozoans, rotifers and crustaceans in ecotoxicity tests (Simonini et al., 2019). Furthermore, as *H. okudai*, the worm emits two types of feeding mucus with paralytic and digestive activities (Kawai et al., 1999; Osman et al., 2010a, 2010b; Iori et al., 2014). To perform these studies, we maintained some specimens of *H. parthenopeia* under laboratory conditions for some months, during which several problems emerged. The management of the tanks was labour-intensive (Simonini et al., pers. Obs.). Given that worms did not consume commercial fish feed (e.g. TetraMin fish flakes, Sera Marin Granulat), they were fed on commercial live *R. philippinarum* (Saito et al., 1999). Every two or three days, the tanks were checked removing the residuals of consumed clams (found with open and clean valves, with or without traces of digestive mucus) to avoid their decay. As in previous studies, many worms were maintained together in tanks filled with a layer of mud-sand (7–10 cm) and sea water, without compartments (Saito et al., 1999; Osman et al., 2010a, 2010b; Iori et al., 2014). With those settings, the handling of sediment to remove the remains of buried clams and collect the worms was time expensive, worsening the quality of water and sediment. Evidence of stress for the polychaetes was also observed: after their manipulation, about half of the worms emitted toxic mucus, the 30% suffered body autotomy and 10% did not recover and died few days later (Simonini et al., personal observation).

The researches of Osman et al. (2010a), Saito et al. (1999) and our experience suggest the potential of culturing worms belonging to the genus *Halla* successfully exploiting clams as feed, but the current systems of worm maintenance and management procedures (e.g., retrieve worms buried in the sediment, prevent water and sediment quality deterioration, removal of clams and their remains) need to be refined to improve the health and growth of the worms. Investigating the appropriate types of diet also constitutes a key step for the optimization of rearing conditions (Olive, 1999; Prevedelli, 1994; Prevedelli and Vandini, 1997; Safarik et al., 2006; Nesto et al., 2012). Moreover, given that in the lab predation events and prey consumption usually occur in the dark and under the surface of the sediment, we did not have the opportunity to assess whether the feeding behaviour of *H. parthenopeia* from the Western Mediterranean resembles that of *H. okudai* from Japan and *H. parthenopeia* from the Suez area.

In the present study, new solutions for the maintenance and growth of *H. parthenopeia* were developed to overcome the problems found in the previous experiences. The foraging behaviour and prey preferences of *H. parthenopeia* were investigated providing specimens of two commercial clam species, *Chamelea gallina* and *R. philippinarum*, as feed to investigate in detail the feeding habits and preferences of a Western Mediterranean population. Then, consumption and growth rates of *H. parthenopeia* feeding on *R. philippinarum* were examined taking into account the effect of both prey and predator size. In addition, intact clams (closed) and artificially opened clams were offered. Up to date there were no information on the ability of *H. parthenopeia* to consume the latter type of resource, which should simulate waste clams. We supposed that the lack of energetic cost for prey handling would lead to an increase in the consumption of open clams, enhancing the growth rate of *H. parthenopeia*. This knowledge could be applied to a small-scale indoor system focused on the exploitation of *H. parthenopeia* as a valuable bait.

## 2. Materials and methods

### 2.1. Management procedures and technical solutions for the maintenance and growth of *H. parthenopeia*

The specimens of *H. parthenopeia* used in our experiments came from sandy-muddy infralittoral sea bottoms (3–10 m depth) off the coast of Marseille, (France), where the benthic fauna is dominated by

polychaetes and veneroid bivalves (Massè and Guérin, 1978). Worms (4–30 g wet weight) were purchased from a retailer as in our previous studies (Iori et al., 2014; Simonini et al., 2019). The worms were transported in their packages (i.e. small plastic boxes filled with 0.5 cm of wet sand containing one or two specimens) placed inside refrigerated containers at 12 °C–17 °C (Iori et al., 2014; Simonini et al., 2019). Once in the laboratory (University of Modena and Reggio Emilia, Modena, Italy), they were temporarily transferred to three 20 l high-density polyethylene (HDPE) flat tanks (60 × 40 cm) filled with about 7 cm of artificial seawater (ASW) and with aeration. ASW was made by mixing deionized water and Reef Crystal, Aquarium System (see Atkinson and Bingman, 1997 for the elemental composition of commercial sea salts). Modena is located inland, making ASW essential for research on marine organisms given the limited availability of natural seawater (e.g. Prevedelli, 1994; Prevedelli and Vandini, 1997; Simonini et al., 2021). The pH (8.0–8.2) and oxygen content (>90% saturation) of ASW were checked routinely during the experiments through a multiparameter water quality meter (Hanna model HI98194). The flat tanks were kept in a thermostatic room equipped with an inverter air conditioner, under controlled conditions (temperature 22 ± 1 °C; salinity 34 ± 1; photoperiod: 12 h light: 12 h dark). The worms were housed in the flat tanks for two-three days to allow acclimatization and assess their health status. The collection from the field, the packaging and the storage cause a huge stress to the worms. Upon opening the package, the worms are almost immobile, but they can recover after 24 h. After 48–72 h it is possible to distinguish “healthy” and “unhealthy” worms. The former show an intact body with a well-developed pygidium or a regenerated posterior end, settled along the edges building tubes of transparent mucus (Fig. 1A,B), and respond to mechanical stimulus (i.e. move toward after a gently stimulation of the pygidium with a tweezer). On the contrary, unhealthy worms show fresh body ruptures, low mobility and responded weakly to mechanical stimulation: they often stayed in the middle of the tank, curling up on themselves. Worms which had autotomised during the acclimatization period were also considered unhealthy.

To simulate an effective small-scale plant for the maintenance and growing of *H. parthenopeia*, healthy worms were moved into experimental tanks. They were located into a recirculating aquarium system housed in the thermostatic room, consisting of eight 90 l glass tanks (total volume: 800 l), and equipped with a skimmer (Bubble Magus HERO180), oxygenators, aquarium heaters and a chiller (Teko TK 500, maximum temperature differential of 0.5 °C). A quarter of the water in each tank was renewed with clean ASW twice a month. Worms were housed individually within transparent polyethylene (PET) containers with 2 l capacity (about 19x16x7 cm length x width x high), internally covered with a plastic sheet, filled with 1 cm of ASW and 5 cm of silica sand (grain size: 79% 1–0.25 mm; 21% 0.25–0.063 mm), and covered with a HDPE net (1 cm mesh) just below the sediment surface (Fig. 1C). After the worms had dug their burrow in the sand, the containers were distributed among the tanks of the recirculation system (Supplementary Material 1).

Previous studies showed that, in both *Halla* species, worms fed under dark conditions. In the case of *H. parthenopeia* from the Suez Canal, specimens were kept in complete darkness, except for experiment set-up, aquaria maintenance and clams checking periods (Osman et al., 2010a). Instead, the experiments on the behaviour of *H. okudai* were performed with light periods from 8:00 AM to 6:00 PM and dark periods from 6:00 PM to 8:00 AM, recording the foraging activity with an infrared video camera during the dark periods (Saito et al., 2004). In our previous experiences we had adopted a 12 h light: 12 h dark photoperiod (Iori et al., 2014; Simonini et al., 2019). In the present study, preliminary tests showed that worms did not respond and fed normally to low-level of red-light illumination (“darkness”, about 15 lx). The incapacity of worm to perceive the red light during “darkness” was assessed turning the light on and off two times and observing their reactions. This trial was repeated ten times in different days when at least one worm in the

aquarium system was engaged in the feeding activity, but no reactions have been observed. Otherwise, in dark conditions, the turning on of a white light (about 50 lx) has always induced the worms to stop foraging, withdraw and remain in their burrows. Therefore, the experiments on *H. parthenopeia* were performed by setting the photoperiod to 12 h “light” (white, intensity about 300 lx, from 6:00 AM to 6:00 PM) followed by 12 h “darkness” (low-level red illumination, from 6:00 PM to 6:00 AM). This procedure allowed the recording of worm behaviour and feeding activity using conventional digital video cameras. Moreover, the presence of sufficient light allowed the operators monitoring the worms and working in the thermostatic room (e.g. for tank checking and maintenance). Given the high sensibility of worms to vibrations, operators must move silently and speak as little as possible in a low voice.

The striped venus clam *C. gallina* and the Manila clam *R. philippinarum* (both belonging to the family Veneridae) were selected as prey. *R. philippinarum* has been introduced and cultivated in several regions worldwide including the coastal lagoons of the Northern Adriatic Sea, which now host some of the European largest clam farms (Bordignon et al., 2021). The native clam *C. gallina* is an economically important species in the Adriatic Sea. It is collected from coastal sandy bottoms between 3 and 7 m (Bargione et al., 2021). Clams were purchased from a local retailer few days before their use and acclimatized in the laboratory with the same procedure and conditions described above. After measuring shell length, the healthy clams (showing unbroken shells and extended siphons) were placed on the surface of the containers randomly. The recirculating system was checked every two/three day to remove and replace the clams consumed by the worms (showing open, almost clean valves, with traces of digestive mucus and soft tissues) or unhealthy clams (showing open valves and tissues still attached but lacking any trace of mucus). Predators were fed *ad libitum*, maintaining prey density at one to two healthy clam per worm. At the beginning and at the end of each experiment, the polychaetes were removed from the sediment and transferred to the HDPE flat tanks described above for checking their health, weight and length measuring (Supplementary material 1).

In addition, a sample ( $n = 12$ ) of unhealthy worms was treated as described above to monitor their survivorship and feeding activity on *C. gallina* for two weeks.

## 2.2. Feeding behaviour of *Halla parthenopeia* on *Ruditapes philippinarum* and *Chamelea gallina*

The feeding behaviour and preferences of *H. parthenopeia* were evaluated through two-alternative trials (Osman et al., 2010a). For each worm ( $n = 16$ ) one specimen of *C. gallina* (length [range size]: 1.9–2.4 cm) and one of *R. philippinarum* (length: 2.6–4.2 cm) were placed in each container. The clams were checked every 24 h and consumed clams were counted and removed. Then, after measuring the shell length, they were replaced with another clam of the same species, maintaining a 1:1 ratio between *C. gallina* and *R. philippinarum*. A digital camera (Sony FDR-AX43) was placed on the top of the aquarium system and worm activity was continuously recorded 24 h/24 for 14 days. At the end of the experiment, further high-definition images and videos were collected at close range with an Olympus TG6 underwater camera to record each step of the feeding activity and prey-predator interactions (Supplementary video 1-6).

The number of *C. gallina* and *R. philippinarum* consumed were used to calculate the daily predation rates and proportions of consumed clams for the two species tested. The latter were compared through a single proportion test and used to calculate the Ivlev electivity index  $E$ , which measures predator preferences *i.e.*, whether a clam species makes up a greater proportion of the diet than the others available (Jacobs, 1974; Saito, 1994).

Daily predation records on forty focal clams were examined to investigate the feeding behaviour and assess the foraging period of the worms, based on the three phases of searching, handling and feeding

identified for *H. okudai* (Imabayashi et al., 1996; Saito et al., 2004). For the population of *H. parthenopeia* considered in this study, the identification of each phase was not always possible. Preliminary analyses have shown that the searching phase was very short (5–10 min) compared to the whole foraging time (up to several hours). Furthermore, in most cases the end of handling and the beginning of feeding were hardly to discriminate because the precise moment in which the worm entered an open shell was not always recognizable. Thus, for each prey-predator interaction, we considered the following phases: 1) start of handling (the time of the first contact between the worm and a clam); 2) end of feeding (the time of the last interaction and definitive leaving of the worm from the consumed clam); 3) duration of the handling + feeding phase (HFP, the time interval between the start of handling and the end of feeding); 4) eventual interruptions, during which the worm came back to its tube and then back again to the prey before the HFP ending. Differences in the duration of the HFP between *C. gallina* and *R. philippinarum* were assessed through a *t*-test after checking for homoscedasticity of the data through a *F* test (significance level 5%).

## 2.3. Preferences, consumption and growth rates of *H. parthenopeia* feeding on *R. philippinarum*

Evaluating the preferences and consumption rate of *Halla* species requires the identification of size classes for both predator (based on worm length or fresh weight) and prey (based on clam shell length) (Saito et al., 2004; Osman et al., 2010a, 2010b). In the present study, the attribution of worms to a specific size class was based on weight instead of length, since the latter is strongly influenced by the contraction/relaxation of the body.

The size of the prey was chosen based on the valve length of *R. philippinarum* specimens consumed in the first feeding behaviour experiment, since the size range of commercial *C. gallina* was very narrow and did not allow for the identification of separate size classes (Supplementary Material 1). As a result, the clams were divided into two size categories, “small” (valve length 3–3.2 cm) and “large” (3.5–3.7 cm). Then, they were kept in two compartments of a separate tank in the aquarium system.

A first experiment was carried out using only healthy clams as feed, with intact shell and closed valve (thereafter “closed” clams or “C clams”). Thirty specimens of *H. parthenopeia* were weighed to prepare six size-groups of five specimens each, which were named according to their mean weight: 5.2 g; 7.0 g; 13.1 g; 14.1 g; 16.3 g; 23.9 g. Each group was placed in a separate experimental tank (replicate,  $n = 6$ ), and each worm in a container. After the worm dug its tube in the sand, one small and one large clam were added on the net in each container. The following day, the consumed clams were counted, removed, measured for shell length, and replaced with new ones of the same size class. This procedure was repeated every 24 h for 30 days (Saito et al., 1999). A 1:1 ratio of small to large *R. philippinarum* was maintained. At the end of the C clams experiment, the worms were removed from the containers and weighed again.

A second experiment was performed to evaluate whether *H. parthenopeia* preferred to feed on closed (C) or artificially opened (O) *R. philippinarum* (O + C clam experiment). O clams simulated wasted bivalves with open or broken valves and residual meat still attached to the shell (Medina Uzcategui et al., 2022). They were obtained by cutting the adductor muscles of C clams with a knife. This procedure ensured greater reproducibility than breaking the shell through mechanical shocks. We expected the worms to recognize the O clams as a profitable food, in which the meat was easily accessible and could be consumed without spending time and resources in handling. Six groups of five *H. parthenopeia* each were defined considering the size classes used in the C clam experiments. The groups were placed in a separate experimental tank (replicate,  $n = 6$ ) and named according to their mean weight: 5.9 g; 7.5 g; 12.5 g; 13.7 g; 17.2 g; 24.2 g. Four specimens of *R. philippinarum* were placed on the net in each container: two C clams

(one small and one large) and two O clams (one small and one large). The consumed clams were treated as described above, while each uneaten O clam (with intact tissue) was removed, discarded and replaced with a new one of the same size class every 24 h to avoid tissue decay. The 1:1:1:1 ratio for the four prey categories was maintained during the 16 days of the O + C clam experiment (Supplementary material 1).

In both experiments, for each size class of the predator, the daily predation rates and the proportions of small and large clams consumed were calculated. The latter were used to obtain the Ivlev electivity index (E). The preference between O and C clams was also evaluated through a single proportion test. The relationships between the predator size class and E were assessed through the Spearman correlation ( $r_s$ ). Since six independent size-groups of *H. parthenopeia* were employed in both C clam and O + C clam experiments, the empiric values of  $r_s$  were considered significant ( $p < 5\%$ ) if greater than or equal to 0.94 (Whitlock and Schluter, 2000). The relationships between the main morphometric traits of prey (i.e., shell length [SL], fresh weight [FWM] and dry weight of the meat) were obtained for a group of 80 *R. philippinarum* specimens through regression models (Supplementary Material 1). The equation  $FWM = 0.0215 SL^{3.40}$  ( $R^2 = 0.92$ ;  $t = 29.97$ ;  $p < 0.0001$ ) was used to estimate the expected FWM by each size group of *H. parthenopeia* for both C and O + C clam experiments. The daily predation rate was expressed in terms of FWM and  $n^\circ$  of clams consumed per day per worm. Since the individuals belonging to each size group were undistinguishable, their weight measures were averaged at group level (experimental tank) at the end of each experiment. Growth was assessed in terms of absolute variation (final weight – initial weight [g]) and relative increase [ $GI\% = (\text{final weight} - \text{initial weight})/\text{initial weight}\%$ ] for each size group of *H. parthenopeia*, and reported daily.

All statistical analyses were carried out with the software PAST (Hammer et al., 2001).

### 3. Results

#### 3.1. Management procedures and technical solutions for the maintenance and growth of *H. parthenopeia*

All the examined unhealthy worms ( $n = 12$ ) did not feed during the two weeks of observation, and only 75% was able to burrow in the sediment, most of which (6/9) died in their burrows. The accumulation of organic material caused the formation of black anoxic zones around the galleries and forced the removal of all the sediment from the PET containers. Otherwise, all the specimens considered healthy (80 worms) survived during the research period (about one year). Their collection from the small PET containers was an easy and quick procedure (supplementary Material 1). The purple mucus emission due to worm manipulation and transfer was very low. All the worms were “healthy” and exhibited a well-developed pygidium and high motility. Autotomy events occurred in  $<10\%$  of manipulations. The HDPE net placed on the container surface did not allow the clams to completely burrow in the sediment, simplifying their detection and replacement after consumption without the need to dig and move the sediment. The sediment in the small containers was always clear: no dark zones were observed even when the amount of food administered was very high as in the O + C experiment. Any worm reaction to the low-level of red-light illumination was observed.

#### 3.2. Feeding behaviour of *Halla parthenopeia* on *Ruditapes philippinarum* and *Chamelea gallina*

The analyses of images and videos obtained with the underwater camera allowed a detailed reconstruction of the three main phases of the feeding behaviour of *H. parthenopeia* (Fig. 2, Supplementary video 1-6). Initially, the worm emerged from the tube and searched for the prey for 5–10 min (Fig. 2A,B). If the clam was too far away from the tube entrance, the worm often grabbed and dragged it closer. Sometimes

*H. parthenopeia* tried to hide the prey and the tube entrance with a cover of sediment mixed with mucus. Often the worms moved from their containers to the adjacent ones (Fig. 2B, Supplementary video 1-3). During handling, the polychaete crawled back and forth along the valve junction, often stopping at the hinge, and emitting an abundant quantity of mucus enveloping the clam (Fig. 2C). This process was repeated several times until the clam valves opened: the larger the prey, the greater the amount of mucus emitted, and the frequency of interruptions observed (Supplementary video 4-6). The feeding phase began when the head of the worm entered the bivalve to consume the meat (Fig. 3D) and ended when the satiated worm left the shell and returned to its tube. Immediately after foraging, shells resulted still enveloped in a gelatinous mass, composed of mucus and digested meat, sometimes interspersed with few fragments of more compact tissues (siphons and foot) (Fig. 2E, F). All the foraging phases are showed in detail in Supplementary Video 1-6.

Overall, the sixteen specimens of *H. parthenopeia* consumed 106 clams in 14 days, 62C. *gallina* (58%, Ivlev electivity  $E = 0.078$ ) and 44 *R. philippinarum* (43%,  $E = -0.093$ ). The difference in proportions of consumed clams for the two tested species was close to significance ( $z = 1.75$ ,  $p > 0.08$ ). The mean daily predation rates on *C. gallina* and *R. philippinarum* were  $0.28 \text{ clam worm}^{-1} \text{ d}^{-1}$  and  $0.20 \text{ clam worm}^{-1} \text{ d}^{-1}$ , respectively. The shell length of clams consumed by *H. parthenopeia* ranged from 1.9 to 2.4 cm and from 2.6 to 3.8 cm for *C. gallina* and *R. philippinarum*, respectively (Supplementary Material 2).

Daily recordings showed that worms were mainly active under dark conditions (Fig. 3A,B, Supplementary Material 2). Indeed, 90% of handling events started between 6:00 PM and 6:00 AM (Fig. 3A), while 68% of feeding activities ended before the period of light (Fig. 3B). The duration of the handling + feeding phase (HFP) was significantly shorter ( $t = 2.762$ ,  $p (0,01)$  for *C. gallina* (mean  $\pm$  standard deviation:  $183 \pm 119$  min) compared to *R. philippinarum* ( $229 \pm 129$  min)). The HFP for *R. philippinarum* was characterized by at least one interruption in  $>70\%$  of the events, while in *C. gallina* the HFP was continuous in about half of the cases (Supplementary material 2). No mortality was observed during the experimental period. Predators were fed *ad libitum*: at every check, at least two living clams were found in each tank.

#### 3.3. Preferences, consumption and growth rates of *H. parthenopeia* feeding on *R. philippinarum*

In the first experiment, where only closed (C) *R. philippinarum* were offered to *H. parthenopeia*, the daily predation rates of small worms (size classes 5.2 g and 7 g) feeding on large clams (valve length 3.5–3.7 cm) were six times lower than those of large worms (size classes 16.3 g and 23.9 g). The proportions of large *R. philippinarum* in the diet were three times higher for large-sized than for small-sized worms (57–68% and 17–20%, respectively). A significant positive correlation ( $r_s = 0.94$ ,  $p < 0.05$ ) emerged between the predator size and its electivity for large-sized prey (Fig. 4A). Opposite trends were observed in the case of small clams (3–3.2 cm), which were the favourite prey of small-sized worms (Supplementary material 2).

When both opened (O) and closed (C) clams were offered (O + C experiment), the predator showed a strong preference for the former ( $n_o = 117$ , 85% of all consumed clam; single proportion test:  $Z = 8.15$ ;  $p < 0.001$ ). The proportions of O clams consumed was the highest for both small (37–48%) and large clams (35–53%), and similar among all the size classes of *H. parthenopeia*. The same trend was observed for the daily predation rates. Accordingly, no significant correlations were found between the predator size and its electivity for large prey (Fig. 4B). The consumption of C clams was always lower than O clam. Notably, the proportion of C clam in the diet ranged from 0% to 15% and from 4% to 9% for small and large clams, respectively (Supplementary material 2).

The individual daily consumption (in terms of fresh weight of meat, FWM) in the O + C clam experiment was higher ( $0.33\text{--}0.42 \text{ g worm}^{-1} \text{ d}^{-1}$ ) than in the C clam experiment ( $0.11\text{--}0.27 \text{ g worm}^{-1} \text{ d}^{-1}$ ). In



**Fig. 2.** Phases of foraging behaviour of *H. parthenopeia* on *R. philippinarum*. A) Healthy clam just placed in the container with extended siphons; B) *H. parthenopeia* moving among the containers looking for a prey (searching phase); C) once reached a prey, the worm crawled several times along the hinge of the shell releasing mucus (handling phase); D) at the opening of the valves, the worm entered the shell and started feeding (feeding phase); after feeding events, the clam results enveloped in the mucus (E) and lacking of residual tissues (F). For details see Supplementary video 1-6.

particular, the FWM consumed by the two small size classes of *H. parthenopeia* was three times higher in the O + C clam experiment (0.42 and 0.36 g worm<sup>-1</sup> d<sup>-1</sup> for 5.9 and 7.5 g groups, respectively) than in the C experiment (0.13 and 0.11 g worm<sup>-1</sup> d<sup>-1</sup> for 5.2 and 7.0 g groups, respectively) (Fig. 5A, Table 1).

For each size class of *H. parthenopeia*, the highest growth rates were obtained in the O + C experiment (Fig. 5B). In particular, the growth rates of the small-sized worms increased up to two-three times in the O + C clam experiment (4,6%-2,9%) compared to the C clam experiment (1,8%-0,8%). In both cases, the lowest growth rates were calculated for the largest worms (C: 23.9 g, 0.1%; O + C: 24.2 g, 0.2%) (Fig. 5B, Table 1).

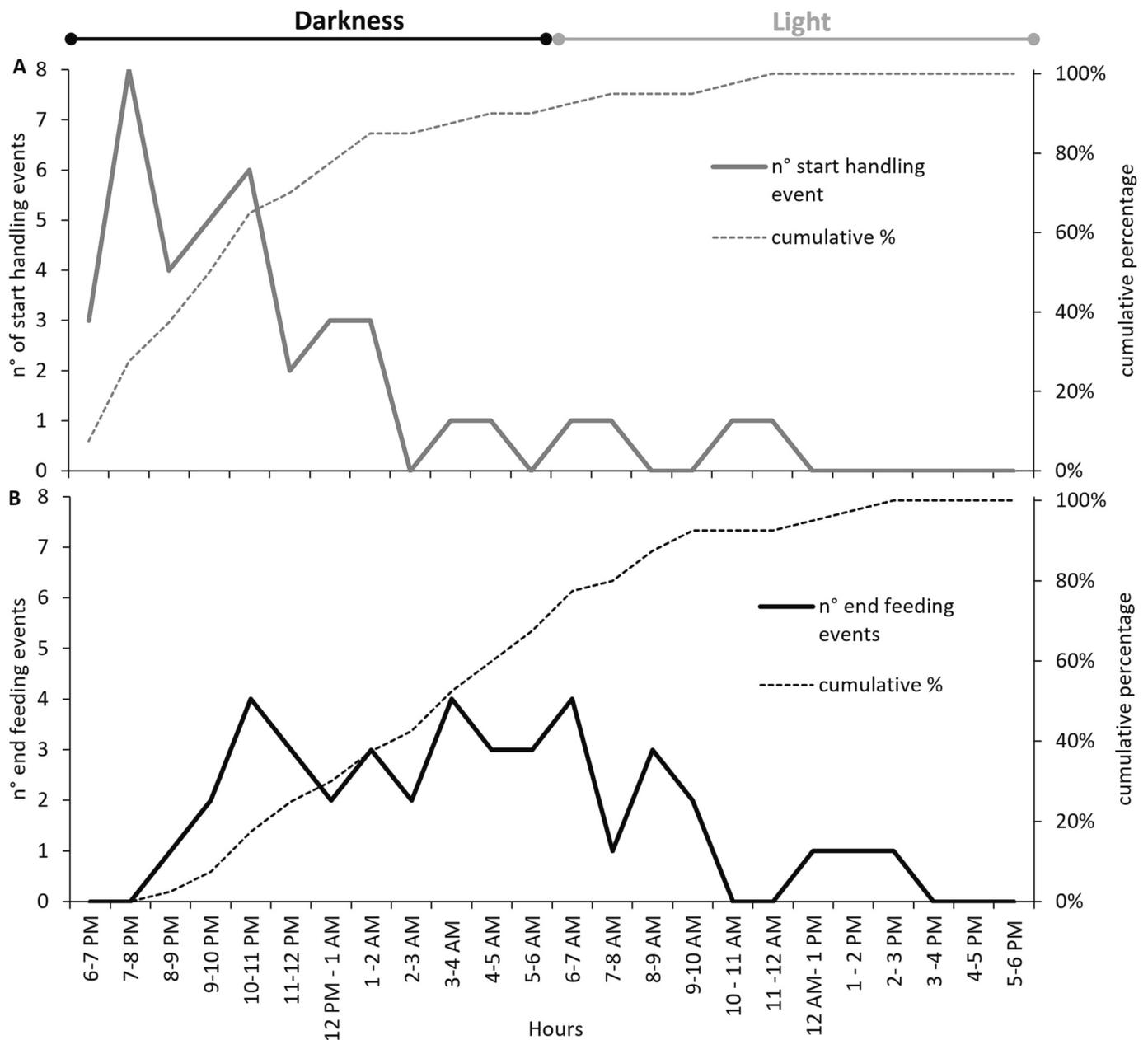
At each check, uneaten clams (at least one O clam and two C clams per tank) were always found. The behaviour of *H. parthenopeia* when feeding on the meat of O clams was like that described for the C clam, except for the handling phase which was completely missing. *H. parthenopeia* normally covered the prey's meat with digestive mucus before consuming it, although in some cases it has been observed that the worm could tear off pieces of tissue directly using its jaws (Supplementary material 1).

## 4. Discussion

### 4.1. Management procedures and technical solutions for the maintenance and growth of *H. parthenopeia*

In the present study several solutions have been developed to

optimize the maintaining and growth of *H. parthenopeia* under controlled conditions and to permit an easy management of the aquarium system even during the execution of the experiments. The process of acclimatization and the selection of healthy specimens of *H. parthenopeia* were essential because if unhealthy worms are collected from the field, they could not recover from the stress. Moreover, the introduction of unhealthy worms in the tanks should be avoided because after dying the decay of their remains can worsen the environmental quality. Experiments focused on the indoor rearing of large sized polychaetes strongly require the setup of a recirculating aquaculture system to optimize worm survival and growth (Nesto et al., 2012; Pombo et al., 2020; Lim et al., 2021). Previous studies on *H. okudai* were performed on single specimens which were often isolated in small containers (1–2l), because the interest was focused on the single individual behaviour rather than on its growth (e.g., Saito et al., 1999, 2004). In the rearing trials of Osman et al. (2010a, 2010b), *H. parthenopeia* was maintained in 13 l PET tank (3 worms each) containing a layer of sediment and connected to a small re-circulating system. In all studies, many worms were maintained together in tanks filled with a layer of mud-sand (7–10 cm) and sea water, without compartments (Saito et al., 1999; Osman et al., 2010a, 2010b; Iori et al., 2014). In the present study, this system was improved housing the worms in small PET containers covered with a plastic net and placed on the bottom of the tanks connected to a recirculating system and a skimmer. These solutions prevented water and sediment deterioration, and allowed an easy removal of clams and their remains. Besides, the separation of worms in small containers simplified their extraction and manipulation and minimized the stress for the worms,



**Fig. 3.** Occurrence of handling + feeding phases and their cumulative proportion observed for *H. parthenopeia*. The beginning of the handling events (a) was more frequent during the dark periods (6 PM-6 AM), while the feeding events ended (b) during the light hours (6 AM-6 PM).

which autotomized very rarely. At the end of the experiments, healthy intact worms with high mobility were obtained, which seem suitable for the commercialization.

**4.2. Feeding behaviour of *Halla parthenopeia***

The present study has extended knowledge on the biology and behaviour of *H. parthenopeia* from the Western Mediterranean. This polychaete was mostly active between 6:00 PM and 6:00 AM, it was not affected by a low-level red illumination and exhibited the same three main feeding phases observed for the congeneric *H. okudai* (i.e., searching, handling, and feeding). When *H. okudai* feeds on *R. philippinarum* close to the entrance of the tube, the searching phase requires from few minutes to three hours (Imabayashi et al., 1996). The duration of handling and feeding phase (HFP) is proportional to prey size: in particular, the consumption of *R. philippinarum* ranging from 2.5 to 4.0 cm in length requires 2–6 h (Saito et al., 2004). In *H. parthenopeia*,

the duration of the HFP observed when worms fed on *R. philippinarum* (length: 2.6–3.8 cm) was also similar, ranging from 1 to 4 h, and was often longer than that required for the small-sized clam, *C. gallina*. Close-range recordings revealed new peculiarity of *H. parthenopeia* feeding behaviour which were not reported in previous study on the genus *Halla*, such as the ability to drag prey near the tube entrance, a characteristic back and forth movement (BFM) along the valve junction of the clam, and the occurrence of interruptions during HFP. The BFM behaviour could represent a strategy for detecting prey size though mechanoreception. Mechanoreceptor cells are described for the epidermis of other polychaetes, but their function is not well understood (Rodrigo et al., 2018). The HFP interruptions occur mainly during handling and their frequency seems correlated with HFP duration and prey size: the larger the prey, the longer it takes for the paralytic mucus to exert its effect (Saito et al., 1999). In the field, worms could be exposed to consumers during the HFP, thus interruptions may represent a defensive strategy: *H. parthenopeia* could remain safe in its tube waiting the valves to open.

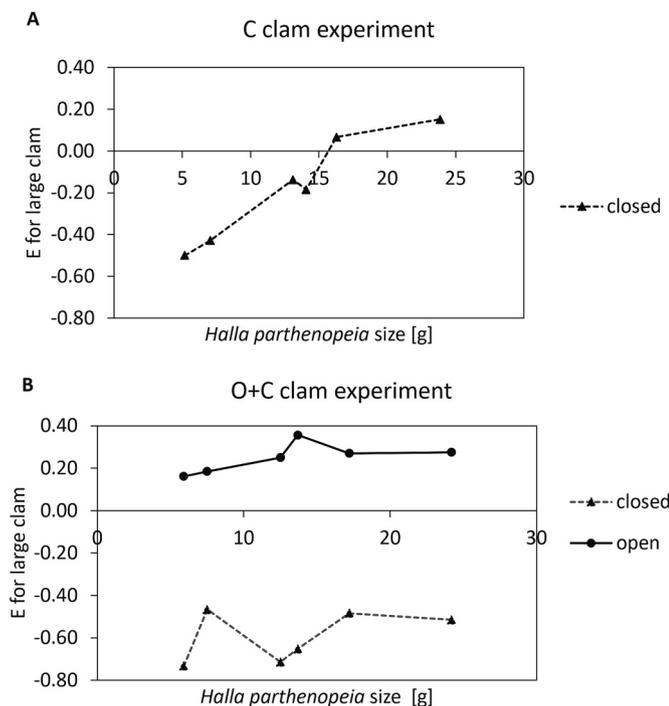


Fig. 4. Electivity (E) for large clams calculated in groups of *H. parthenopeia* with different size in the C clam (A, only “closed” clam provided) and O + C clam (B, both “Open” and “closed” clam provided) experiments.

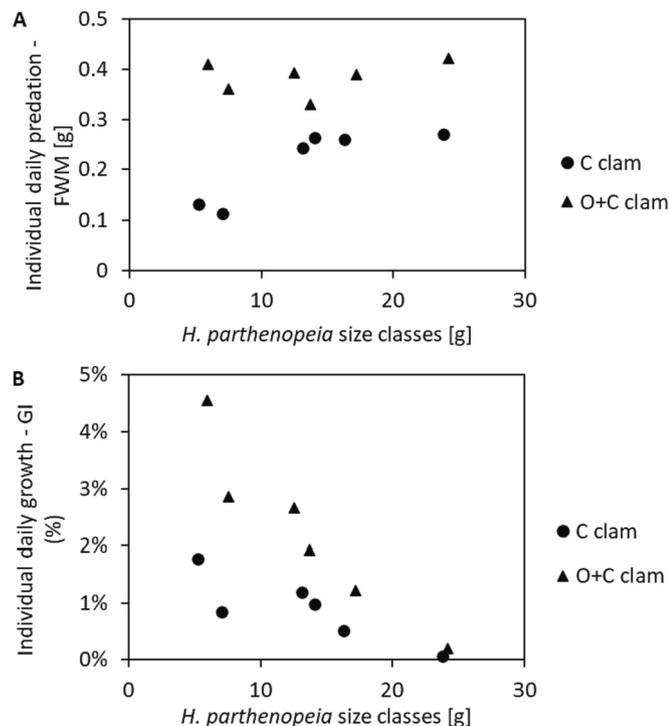


Fig. 5. Individual daily predation rate for each size group of *H. parthenopeia* in the C clam and O + C clam experiments, expressed in terms of A) fresh weight of clam meat (FWM) consumed daily by each worm, and B) daily growth increment.

Indeed, the behaviour of palatable worms such as *H. parthenopeia* appears often constrained by predation risk: they forage when consumers are less active, tend to live in structural refuges burrowed in unconsolidated sediments, and are a mobile, nocturnal species (Kicklighter and

Table 1

Predation rates expressed as number of consumed clam (DPR) and fresh weight of the meat [FWM] consumed per individual per day, and daily mean growth in terms of variation (final weight – initial weight [g]) and growth increment [GI% = (final weight – initial weight)/initial weight%] for each size group of *H. parthenopeia* in the C clam (duration: 30 d) and O + C clam (duration: 16 d) experiments.

C clam experiment					
<i>H. parthenopeia</i> average group weight [g]		Predation rates		Mean daily growth	
initial	final	DPR [Clam worm <sup>-1</sup> day <sup>-1</sup> ]	FWM [g worm <sup>-1</sup> d <sup>-1</sup> ]	Variation [g]	GI (%)
5.2	7.9	0.12	0.13	0.09	1.8%
7.0	8.8	0.10	0.11	0.06	0.8%
13.1	17.8	0.19	0.24	0.16	1.2%
14.1	18.2	0.21	0.26	0.14	1.0%
16.3	18.8	0.19	0.26	0.08	0.5%
23.9	24.4	0.19	0.27	0.02	0.1%

O + C clam experiment					
<i>H. parthenopeia</i> average group weight (g)		Predation rates		Mean daily growth	
initial	final	DPR [Clam worm <sup>-1</sup> 1 day <sup>-1</sup> ]	FWM [g worm <sup>-1</sup> d <sup>-1</sup> ]	Variation [g]	GI (%)
5.9	10.2	0.33	0.41	0.27	4.6%
7.5	10.9	0.28	0.36	0.21	2.9%
12.5	17.8	0.30	0.39	0.33	2.7%
13.7	17.9	0.24	0.33	0.26	1.9%
17.2	20.6	0.29	0.39	0.21	1.2%
24.2	25.0	0.31	0.42	0.05	0.2%

Hay, 2006).

#### 4.3. Preferences, consumption and growth rates of *H. parthenopeia* feeding on bivalve biomass

Selective predation based on prey size can significantly impact the composition of benthic communities (Sanchez-Salazar et al., 1987; Grosholz et al., 2000). *H. okudai* and *R. philippinarum* were used as a model to investigate the role of prey- and predator size in shaping predator behaviour and preferences in the case of infaunal invertebrates (Saito, 1994; Saito et al., 2004). Large *H. okudai* specimens (10–23 g) show higher electivity for large clam (3.0–4.0 cm valve length) than for small (1.0–2.0 cm) ones, even when encounter rates are the same. The opposite relationship is observed in small-sized *H. okudai* specimens (0.3–2 g), which prefer small prey (Saito et al., 2004). In the present study, the use of commercial clams and the predator’s lack of interest for clams which exceeded 3.8 cm in length, resulted in a reduction of the size range of prey considered (3.0–3.7 cm). This forced the adoption of only two size classes of clams in the experiments on predator-prey preferences. Nevertheless, the use of six size classes of *H. parthenopeia* (from about 5 to 24 g weight) allowed to identify clear preference trends consistent to those reported for *H. okudai*. Our findings confirmed that, when handling is required (offering only intact, closed prey, C clam experiment), a significant positive correlation occurs between predator size and its electivity for large-sized prey. Opposite trends were observed for the consumption rates and preferences for small-sized clams. This finding supports that small polychaetes have an advantage when selecting small prey, which require less time and energy for handling and feeding than large prey (Saito et al., 2004). As expected, the worms recognized O clams as attackable prey. When both artificially opened (O) and closed (C) clams were offered (O + C experiment), *H. parthenopeia* preferred to feed on the former, regardless of the size of prey and predator, leading to the loss of the correlation between prey

and predator size. Small worms also attacked large O clams, since they resulted in the most profitable prey without energy costs related to handling. The results confirmed the importance of the cost of prey manipulation in shaping the feeding preferences in *Halla* species (Saito et al., 2004; Osman et al., 2010a, 2010b), and demonstrated for the first time that the handling phase is not necessary to trigger prey consumption. Probably the commercial feeds tested in our previous studies, which also do not require manipulation, were not consumed because they were not recognized as a suitable feed.

To date, the only information on *H. parthenopeia* consumption rates and growth have been obtained by Osman et al. (2010a, 2010b) examining a population from the Suez Canal. When fed on the venerid clam *Paphia undulata*, the worms (average weight 12.9, 18.5, 23.4 g) showed individual daily consumption rates (ICR) ranging from 1.73 to 2.57 clam worm<sup>-1</sup> d<sup>-1</sup>, and growth increment rates (GI) ranging from 0.04 to 0.08 g worm<sup>-1</sup> d<sup>-1</sup>. The increase of predator size has been associated with ICR decrease and GI increase (Osman et al., 2010a, 2010b). In the rearing trials on *H. okudai* fed on *R. philippinarum*, worms weighting 12–13 g consumed 0.4 clam worm<sup>-1</sup> d<sup>-1</sup> (FWM = 0.43 g worm<sup>-1</sup> d<sup>-1</sup>) with a GI of 0.13 g worm<sup>-1</sup> d<sup>-1</sup> (Saito et al., 1999). However, all these studies were performed by offering only intact, closed clams to the worms. Here, *H. parthenopeia* feeding on C clam showed GI rates similar to those found in previous experiments, while the ICR was 2–10 times lower. The relationships between worm size and ICR, and worm size and GI, were maintained: in particular, small-sized worms attained higher growth rates than larger ones. Maximizing the growth rate should be a primary target for small animals which may be more vulnerable to consumers while foraging and have less chance of survival (Osman et al., 2010a, 2010b).

When open clams were also offered (O + C clam treatment), the ICR increased reaching values like those reported for *H. okudai*, while the GI triplicated. The removal of the handling phase induced an increase and a homogenization in the ICR for all the size classes, and a dramatic increase in the GI in the small-sized *H. parthenopeia*. Perhaps, small worms may have highest metabolic rates and low maintenance cost. Thus, they could efficiently benefit from the high amount of resources available in the O + C clam experiment. A decrease in GI with age and body size is commonly reported in experiments where polychaetes are fed *ad libitum* with high nutritional food (Nesto et al., 2012). Perhaps, in addition to the supply of open clams, the new management procedures and technical solutions also contribute to the increase in the ICR and GI rate observed, compared to those of the previous studies (Osman et al., 2010a, 2010b; Saito et al., 1999).

#### 4.4. Feasibility of a small-scale indoor grow-out system for *Halla parthenopeia*

A small-scale indoor plant for the maintenance and growth of *H. parthenopeia* fed with clam waste could be useful for *H. parthenopeia* collectors and sellers helping to meet the demand of the angling community for large, high-quality worms. Based on the GI values, when feeding on O clams, a 5-g worm (the smallest marketed size, about 3 € each) can grow up to 20 g (about 13 € each) in just two months, reaching four times the initial economic value. Furthermore, all the worms left unsold at the end of the most favourable fishing period (May–October, Baeta et al., 2019) can be kept in the system, surviving and growing until the following season, also reducing the need to collect new specimens from the field. Except for worms which were sacrificed to perform histological and transcriptomic analyses, all the specimens used in the experiments have survived to this day (October 2023). Given that *H. parthenopeia* specimens were purchased in autumn 2021, they can be healthily reared indoor up to two years. Our results showed that *H. parthenopeia* can feed on two clam species, *C. gallina* and *R. philippinarum*, which have high economical relevance and are available from local fishermen, farmers and/or retailers throughout the year. However, a diet based on commercial clams is very expensive (retailer

price: 12–16 € kg<sup>-1</sup>, *R. philippinarum*, Italy, March 2023). Given that an aquaculture system should promote a cheaper production than current market prize, commercial bivalves seem thus unaffordable to rear *H. parthenopeia* apart from research purposes. However, our results suggested that *H. parthenopeia* can grow quickly when fed with opened clams, simulating bivalve waste containing residual meat. The industrial processing of bivalves (clam, oysters, and mussels) produces significant quantities of wastes. For example, in Italy, the farming of *R. philippinarum* generates >30,000 tons (32.3%) of clam waste (Corbau et al., 2023), comprising shells and residual meat. Commercial bivalve waste biomass includes also the ones produced by fisheries (such in the case of *C. gallina*), commercial retailers (e.g., unsold clams from supermarkets) and mussel farms. The employment of meat from waste clams may reduce the costs for worm rearing and permit the exploitation of *H. parthenopeia* both as a bait and a source of bioactive molecules. Indeed, although a wide array of natural products from polychaetes with biotechnological potential has just come into focus, fewer than 200 compounds have been isolated during the last century (Coutinho et al., 2018; Simonini et al., 2019; Righi et al., 2022). The defensive and feeding mucus produced by *H. parthenopeia* may be of biotechnological interest due to their cytotoxic and paralytic activity, respectively. Besides, the maintaining of worms under controlled conditions could provide new insights into the fundamental aspects of *H. parthenopeia* biology required for its broad-scale culture.

## 5. Conclusion

In the present study, new advantageous technical solutions and management procedures for the maintenance and growth of the polychaete *H. parthenopeia* in small, indoor rearing system were developed. We described the feeding behaviour of Western Mediterranean population of *H. parthenopeia*, including the details of the process of clam manipulation which have not been previously reported for the genus. We found out for the first time that *H. parthenopeia* recognize open clams as profitable prey, and a diet based on this resource could increase the growth of small specimens up to 2–4 times more than a diet based only on closed clams. Given the high commercial value and biotechnological potential of *H. parthenopeia*, these new knowledges could be easily applied to a small-scale niche production focused on the maintenance and growth of worms fed with clam waste.

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## CRedit authorship contribution statement

**Anita Ferri:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Sara Righi:** Methodology, Writing – review & editing. **Daniela Prevedelli:** Writing – review & editing, Resources. **Roberto Simonini:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Funding acquisition, Resources, Supervision.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Simonini Roberto reports financial support was provided by National Recovery and Resilience Plan (NRRP).

## Data availability

Data will be made available on request.

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