

Comparative study of the gut contents of *penaeus japonicus* Bate 1888 (Decapoda: Penaeidae) in semi-intensive culture and in brackish water wild environment

Abstract

The trophic adaptability of a species may influence its dispersion potential and the ability to invade foreign territories. Understanding the factors that facilitate trophic adaptability may help the provision of forecasts about the potential dispersion of allochthonous species, even in a warmer and acidified world, according to the current trends of global changes. Various studies demonstrated the adaptability of *Penaeus japonicus* Bate to variable feeding regimes under natural conditions. To optimize artificial diets for the aquaculture of Penaeid shrimps, gut content data of specimens cultured in ponds were compared to contents from shrimps fed on natural macro benthic communities in a brackish-water lagoon. In addition, the feeding adaptability of this shrimp to scarcely diversified benthic associations was tested in aquaculture ponds. Our comparative analyses confirm that *P. japonicus* feeding pattern may be largely adapted to variations in the available benthic organisms, in different management conditions.

Keywords: *Penaeus japonicus*, Food, Brackish water, Culture, Adaptability, *Marsupenaeus japonicus*

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Introduction

Penaeus japonicus Bate 1888, the “Kuruma shrimp”, whose taxonomical identity is still debated Tsoi et al.¹ is an ideal species for aquaculture purposes, due to its adaptability to different environmental conditions Lumare.² The species is still indicated by means of two scientific names, i.e., *P. japonicus* and *Marsupenaeus japonicus*, due to a controversy in the status of the genus *Penaeus* Fabricius, 1798 (see, for example, Pérez Farfante & Kensley Ma et al.^{3,4} Both names are still used by various organizations and databases, eg. WoRMS (World Register Marine Species), FAO, DAISIE (Delivering Alien Invasive Species Inventories for Europe); however, in this study we will follow the most updated results Tsoi et al.¹ for the definition of *Penaeus* and hence we will use the current name *Penaeus japonicus* Bate 1888.

Penaeus japonicus exhibits attractive brownish-red bands and it is named “the kuruma shrimp” because of a characteristic wheel-like banding pattern. It is economically important for the world shrimp market due to an easy management of pond cultures and the fast grow-out.⁵ Many aspects of its physiology and reproductive biology have been clarified.^{6,7} but the available information on its trophic needs in intensive and semi-intensive culture conditions is still incomplete. Previous studies identified the feeding patterns of *P. japonicus* under natural conditions, after its introduction in coastal lagoons.⁸⁻¹¹ In fact, this species appears to be able to easily invade new territories when accidentally introduced in natural environments.¹²⁻¹³ and the global increase of temperatures in natural basins, even in the Mediterranean area, facilitates its dispersion.¹⁴

At present, *Penaeus japonicus* is widely distributed in Japan, the South China Sea, Korea, the Archipelago of Malay, the Red Sea, the northern coast of Queensland in Australia, and the western Indian Ocean, up to the eastern South Africa.³ However, according to Galil &

Zenetos.¹³ it was considered as a Lessepsian migrant in the Mediterranean waters since 1924. Obviously, its successful adaptation in the Mediterranean may threaten indigenous species, as *P. keratourus* and other crustacean decapods, as well as its main prey, represented by molluscs and polychaetes.¹⁵

A dynamic feeding model was demonstrated for this species in relationship to the predation on natural benthic populations.¹⁶ This evidence allows us for predicting a destructive influence of *P. japonicus* on wild ecosystems as well as a certain degree of adaptability to benthic communities characterized by low species diversity, as those characterizing the culture ponds. Therefore a study comparing natural feeding patterns with those observed in prawns managed in extensive and semi-intensive conditions was performed by means of gut contents analyses. The results of the present study, in fact, may allow from one side an optimization of inexpensive diets for this species in aquaculture ponds and, on the other side, to explain some of the factors promoting the adaptability of a given species when introduced in new areas.

Materials and methods

Five ponds were set in Cammarata (Lesina, Southern Italy) (Figure 1). The first 4 ponds (100 m² each one) were managed in semi-intensive conditions, at density of 2.5; 1.5; 2.5; 1.5 post larvae (PL22) per square meter, respectively; the fifth one (200 m²) was managed in extensive conditions, at a density of 12 post-larvae (PL22) per square meter.

Water was partially changed daily, by pumping from the adjacent lagoon, in order to promote correct environmental conditions for the grow-out of the shrimps.¹⁷ and the development of benthic fauna and flora driven by larvae and various organisms present in the lagoon. The finest rate of changes was determined by measuring selected

chemical and physical parameters, i.e., temperature, salinity, pH, dissolved oxygen, dissolved nutrients. In particular, changes were performed to assure consistence of the above-mentioned parameters in comparison to the natural ones, measured in the lagoon.¹⁸ To perform the gut content analyses, three collections of shrimp were obtained in the early night from each pond, to obtain individuals containing undigested prey. The first sample refers to shrimps aged 6 weeks, the second one refers to shrimps aged 12 weeks, and the last one refers to adults sampled a few days before the end of the experiment.

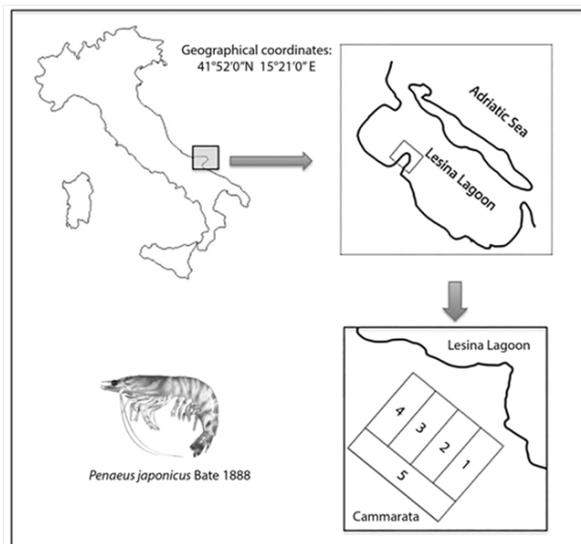


Figure 1 Pond location in “Cammarata”, near the Lesina Lagoon.

Samples were immediately transferred in the laboratory and deep-frozen. Each specimen was measured (total length), weighed, and finally sacrificed, fixed and dissected for the analysis of the gut contents. A presence-absence matrix was compiled, taking into account each specimen sampled and each food item identified in its stomach, according to Zupo et al.² Data were managed by means of a MS-Excel spreadsheet and compared with those obtained by previous authors.^{9,11,16} using Graph pad Prism software. In particular, the following indices were taken into account:

Numeric frequency, obtained on the basis of the following relationship

- $$Fn = (N_{pi} / T_p) \times 100$$
, where

N_{pi} = number of individuals of the food item “i”

T_p = Total number of individuals, of all food items, found in the gut.

Prey frequency, obtained on the basis of the following relationship

- $$F_p = (N_{spi} / N_s) \times 100$$
, where

N_{spi} = number of guts containing the item “i”

N_s = number of “non-empty” stomachs studied.

Vacuity coefficient, obtained on the basis of the following relationship

- $$C_v = (N_v / N_t) \times 100$$
, where

N_v = number of empty guts recorded.

N_t = total number of guts studied.

Previous authors (Piscitelli & Scalera Liaci, 1983) classified each prey, on the basis of their frequency index (Fp), in the following categories:

- $F_p > 50\%$: preferential prey, defining the feeding pattern of the species.
- $10\% < F_p < 50\%$: secondary prey, representing a diet integration.
- $F_p < 10\%$: occasional prey, having little importance in the diet.

In order to obtain quantitative information about the trophic needs of shrimp of different ages, the feeding index “FI”¹⁹ was also calculated; it is computed on the basis of the following relationship:

- $$FI = (PCS / PTA) \times 100$$
, where

PCS = weight of the gut contents.

PTA = weight of the animal body.

Results

The average total length of individuals sampled in all ponds, in the three samples were, respectively 9.4, 13.4 and 15.0 cm (Table 1). The analysis of the gut contents, classified according to larger food categories (Table 2) permits a direct comparison with the data obtained in brackish water wild environments.¹¹ The preferential food items in all samples were Annelid Polychaetes (Fn=16.7%), insect larvae (Fn=12.7; represented mainly by Chironomid Dypters), Molluscs (Fn=15.3), and Crustaceans (Fn=19.9; represented mainly by Amphipods and Copepods). Large amounts of artificial food (Fn=12.4) were found in prawns cultured in semi intensive conditions. Other food items, less important, were macroalgae (Fn=14.0), Foraminiferids (Fn=1.3) and Isopod Crustaceans (Fn=1.3).

Table 1 Main biometric and trophic parameters of the three samples investigated

	Sample 1	Sample 2	Sample 3
Mean length	9.39	13.42	15.05
Mean weight	5.72	15.72	25.19
Gut weight	0.05	0.13	0.16
Cv	13.3	12.5	25.9
FI	0.55	0.18	0.08

The comparison of the numeric frequencies of the main taxa found in the gut contents of individuals sampled in culture ponds (present study) and in natural environments Piscitelli & Scalera Liaci.⁹ demonstrated that the trophic importance of some taxa changed dramatically under different environmental conditions, according to the age of prawns. In particular, insects and polychaetes were clearly prevalent food items in culture conditions, while a higher variability in prey taxa was observed in natural conditions. Molluscs (mainly bivalves) were a preferential prey in both natural and culture conditions. A few taxa were found in the guts of individuals collected in pond algae were represented by four species, i.e., *Blidingia* sp, *Cladophora* sp, *Chaetomorpha gracilis* and *C. capillaries*, polychaetes were represented only by *Polydoraciliata*, bivalve Molluscs were represented by *Cerastoderma glaucum*, *Abraovata* and, at lower abundance, by *Mytilastermarioni* a few gastropods (*Hydrobia* sp) were found in the gut contents of cultured individuals; amphipods were represented by *Gammarusa equicauda* and, more rarely, by *Corophium insidiosum* isopods were represented by a few individuals of Sphaeromids.

The shrimp feeding activity showed a decrease in the last sample. FI values of 0.55, 0.18 and 0.08 were recorded through the three sam-

ples, respectively (Table 1), according to the mean temperatures of 28.5, 26.7 and 17.9, measured during the experimental period. Chironomid dypters accounted for the highest Fp values (88.9%) calculated on the total sampled individuals (Table 2), followed by Polychaetes (61.1%) and Molluscs (55.6), in agreement with the results obtained in the wild by Piscitelli & Scalera Liaci.⁹ The vacuity coefficient was very low (13.3%, 12.5% and 25.9% in the 3 samples) in comparison with the results of previous authors and it is close to the frequency of individuals in ecdysis. A decrease of FI and an increase of Cv indices were detected during the grow-out period in all ponds (Figure

2). Striking differences were observed among the prey frequencies measured in this study (in culture ponds) and in the natural environments. For example, algae, Annelid polychaetes and insects exhibited significantly higher frequencies in the guts of cultured animals in comparison to the wild, while mollusks and crustaceans showed more complex patterns and higher numeric frequencies in the wild (Table 2). However, the abundance of various taxa varied with the age, as younger individuals preferred Crustaceans and small mollusks, while older individuals fed mainly on macrophytes and annelid polychaetes (Table 3).

Table 2 Prey frequency (Fp) and numeric frequency (Fn) data of gut contents of *Penaeus japonicus* cultured in Cammarata, compared to those of populations living in the Lesina lagoon

Prey categories	Fn		Fp	
	This study	Lagoon	This study	Lagoon
Foraminiferids	1.3	1.1	5.6	5.5
Anellides	16.7	7.2	61.1	22.9
<i>Perinereis cultrifera</i>		2.3		
<i>Polydora ciliata</i>	13.9		59.2	
<i>Nereidae</i>		4.2		
<i>Polynoidae</i>		0.7		
Others	2.2		9.2	
Molluscs	15.3	26.5	55.6	43.1
<i>Hydrobiasp</i>	1.3	10.3	3.7	
<i>Cerastoderma edule</i>		3.4		
<i>Cerastoderma glaucum</i>	2.6		11.1	
<i>Haminea navicula</i>		0.9		
<i>Abra ovata</i>	11.3		48.1	
Crustaceans	19.9	32.4	74.1	48.2
<i>Ostracodes</i>		0.1		
<i>Misidaceans</i>		0.1		
<i>Copepods</i>	7.4		31.5	
<i>Amphipodes</i>	11.3		48.1	
<i>Corophium acherusicum</i>		0.4		
<i>Corophium insidiosum</i>	1.3	10.7	7.4	
<i>Gammarides</i>	10.6	0.7		
<i>Gammarusa equicauda</i>	10.6	2.3		
<i>Microdeutopus gryllotalpa</i>		1.5		
<i>Isopods</i>	1.3		3.7	
<i>Spheromides</i>	1.3		3.7	
<i>Cyathuracarinata</i>		6.4		
<i>Idotea baltica</i>		1.4		
<i>Cymothoidae</i>		0.1		
<i>Decapods</i>				
<i>Palaemonetes antennarius</i>		0.9		
<i>Palaemon elegans</i>		0.2		
<i>Palaemonidi</i>		0.3		
Insects	21.7	12.5	88.9	18.3
<i>Chironomides</i>	20.8	12.5	88.9	
Others	1.3		5.6	
Eggs	1.3		5.6	
Fish		18.3		36.2
<i>Syngnathus abaster</i>		7.2		
Macrophytes	14		42.6	6.4
<i>Blidingiasp</i>	1.7		5.6	
<i>Cladophorasp</i>	4.7		20.4	
<i>Chaetomorpha gracilis</i>	4.7		20.4	
<i>Chaetomorpha capillaris</i>	2.6		11.1	
Artificial foods	12.4		74.2	

Table 3 Numeric frequency (%) of taxa recorded in the gut contents of *Penaeus japonicus* at different size classes. Data from the present study are compared to those obtained by previous authors Piscitelli & Scalera Liaci.⁹ in the Lesina Lagoon

Lesina Lagoon	This study					
	10 cm	12-14 cm	14-16 cm			
Size	10 cm	12-14 cm	14-16 cm	10 cm	12-14 cm	14-16 cm
Foraminiferids	22.2	1.9	3.4		1	1.2
Anellides	16.7	25	23.7	11.3	16	20.7
Crustaceans	50	50	44.1	29	14	20.7
Molluscs	44.4	46.2	37.3	25	15	10.3
Chironomids	16.7	13.5	27.1	27.2	20	22.1
Fish	23.3	50	33.9	-	-	-
Macrophytes	-	5.8	11.9	6.8	16	15.6
Artificialfoods	-	-	-	-	17	7.8

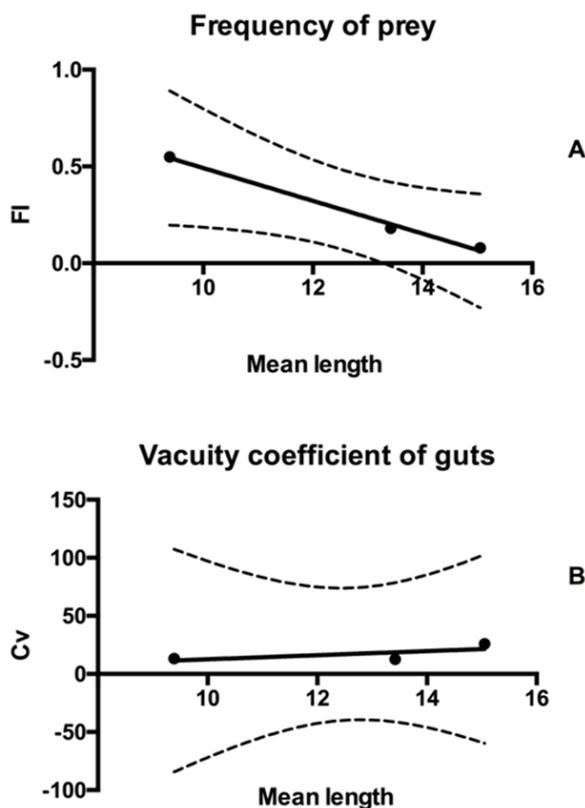


Figure 2 Comparison between the feeding indices considered in the present study; a progressive decrease of the FI index (A) and a slight increase of Cv index (B) is shown, according to the size of the prawn.

In general, Crustaceans and mollusks were more abundant in field-collected shrimps, while insects and annelids were more abundant in cultured individuals, that also exhibited an almost equal abundance of the main food items (Figure 3). The prey frequency index also exhibited variations according to the size when the shrimps collected in the lagoon of Lesina were compared to those collected in pond (Figure 4). In particular, Foraminiferids were almost absent in the cultured shrimps while they were abundant in the youngest individuals collected in the wild. Chironomids were fed especially by larger individuals in the field, while they were used by all size classes in the pond cultures. Macrophytes were mainly consumed by older individuals in the field and their consumption increased, in the tanks, also in younger shrimps (Figure 4a&4b).

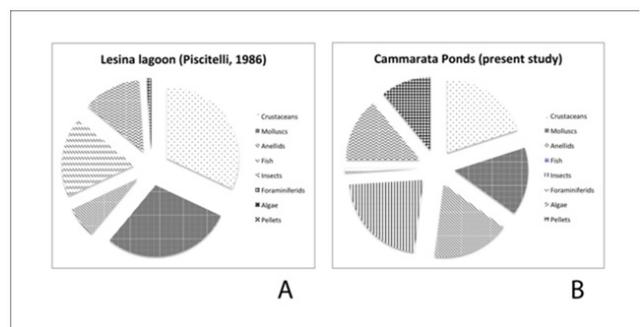


Figure 3 Comparison between the feeding patterns of *Marsupenaeus japonicus* in the wild Piscitelli,¹¹ and b) in culture conditions.

Discussion

The qualitative analyses of the gut contents confirmed the “opportunistic-carnivore” feeding habit of *Penaeus japonicus* and, in general, the adaptability of various dietetic regimes characterizing penaeid shrimps.²⁰ however, its feeding preferences varied mainly according to the age.²¹ The decrease of the feeding activity (FI index) observed in this study was probably due more to the decrease in temperature.²² than to the decrease of the abundance of benthic prey. In fact, the presence of an artificial food should replace any lack of prey.²³ F_n and F_p indices calculated in the present study, compared to those obtained for individuals sampled in the wild, showed different feeding patterns (Table 2). A comparison of the data collected in the present work for three size classes (corresponding to the three subsequent samples), with those of similar size classes sampled in the natural environment.^{9,24} demonstrates prey preference exhibited by prawns in natural conditions, as Crustaceans, Polychaetes and fishes were the most consumed categories. They were substituted by insects, Molluscs and artificial foods (pellets) under culture conditions, as demonstrated in this investigation.

However, it was demonstrated that Polychaetes are important also for the reproduction of this species.²⁵ and therefore the presence of these prey items could be fundamental in culture ponds. Foraminiferids were an important food item for young individuals sampled in the natural environment.⁹ However, they were very quite rare in the guts of cultured individuals. This difference may be due to the low abundance of this prey in the benthic population of the culture ponds. A similar conclusion is reached when prey fish data are analyzed fish were consumed by wild individuals, but they were absent in the culture ponds. These relationships confirm that *P. japonicus* adapts easily to any environment and it is able to exploit low-diversity benthic food associations, as those observed in culture ponds.

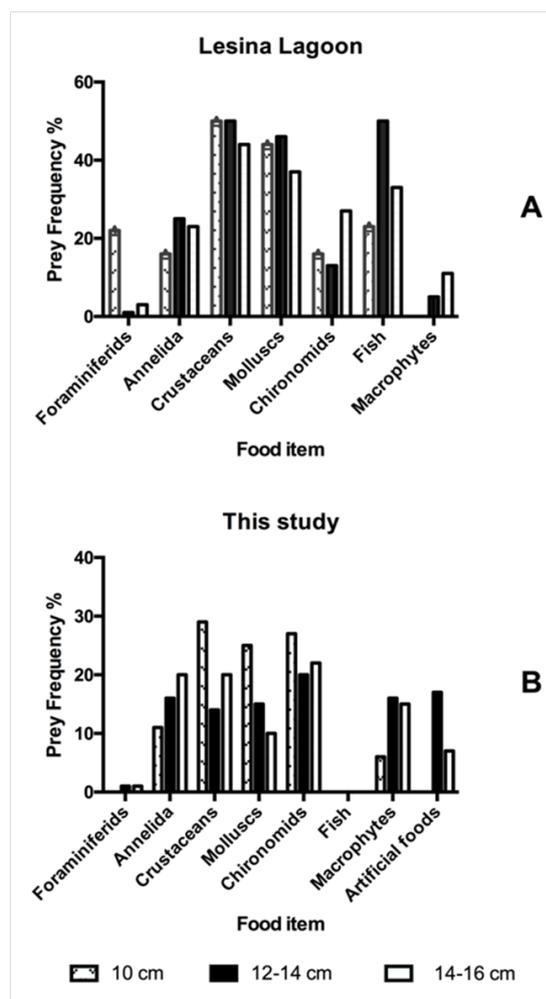


Figure 4 Gut contents of *Marsupenaeus japonicus* according to three age classes. Comparison between the data from A) specimens collected in the Lesina Lagoon Piscitelli & Scalera Liaci⁹ and B) those of the present study.

Arthropods and Molluscs could play an important role in the trophic ecology of this decapod, especially at larger sizes of the prawn; young individuals (< 10 cm total length) cultured in semi-intensive conditions showed a higher preference for Chironomid insects and algae, than individuals sampled in the wild. The frequency of Chironomid insects in the guts of specimens in culture conditions, higher than in natural conditions, could result from an higher abundance of these items in culture ponds; however, these items should be considered as “preferential prey” (Fp>50%). It is worth to observe that also “non-living” organic matter can be exploited by the prawn, given the high values of feeding indices yielded by pellet foods (Fn= 12.4; Fp= 74.2).

Therefore, *P. japonicus* is adaptable to different feeding regimes.²⁶ and it can exploit prey found in a range of environmental conditions. Interestingly, several aspects of its feeding biology were based on single or a few observations.²⁷ As demonstrated by the present study, its dietetic pattern is complex and not constant during the development, but variable also according to the culture conditions. This conclusion confirms the importance of *P. japonicus* for aquaculture purposes and its potentially destructive power for the wild environments. In fact, the ability to exploit many food items may facilitate its life in culture ponds, but it may represent as well a danger for the stability of native

macro benthic associations when *P. japonicus* is accidentally introduced into the wild.²⁸

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Conflicts of interest

None.

References

1. Tsoi KH, Ma KY, Wu TH, et al. Verification of the cryptic species *Penaeus pulchri caudatus* in the commercially important kuruma shrimp *P. japonicus* (Decapoda: Penaeidae) using molecular taxonomy. *Invertebrate Systematics*. 2014;28:476–490.
2. Zupo V, Lumare F, Bisignano V, et al. Ruolo delle associazioni ibentoniche nell' allevamento semi-intensivo di *Penaeus japonicus* Bate: relazioni ponderali. *Nova Thalassia*. 1989;10(1):297–308.
3. Pérez Farfante I, and Kensley B. Penaeoid and sergestoid shrimps and prawns of the world. Keys and diagnoses for the families and genera. *Mémoires du Muséum national d'Histoire naturelle*. 1997;175:1–233.
4. Ma KY, Chan TY, and Chu KH. Refuting the six-genus classification of *Penaeus* s. l. (Dendrobranchiata, Penaeidae): a combined analysis of mitochondrial and nuclear genes. *Zoologica Scripta*. 2011;40(5):498–508.
5. Chan TY. Shrimps and prawns. The living marine resources of the western central Pacific. In: *Carpenter KE & Niem VH (Eds.), FAO Species identification guide for fishery purposes*. USA. 1998.
6. New MB. A bibliography of shrimp and prawn nutrition. *Aquaculture*. 1980;21:101–128.
7. Lumare F. Marine shrimp culture in the world and present state and trends of Kuruma prawn culture in Italy. *Inf Tecn Inst Invest Pesq*. 1986;136:3–23.
8. Tournier H. Conditions d'acclimatation des crevettes *Penaeus kerathurus* et *Penaeus japonicus* dans les eaux du littoral languedocien. *Science et Pêche Bull Inst Pêches Marit*. 1972;213:1–13.
9. Piscitelli G, Scalera Liaci L. Preliminary findings about feeding patterns of *Penaeus japonicus* Bate in the Lagoon of Lesina (Southern Italy). *Oebalia IX NS*. 1983;55–64.
10. Lumare F, Palmegiano GB. Acclimatazione di *Penaeus japonicus* Bate nella Laguna di Lesina (Italia Sud-Orientale). *Riv It piscic Ittiop XV*. 1980;(2):53–58.
11. Piscitelli G. Relazione sull'attività di ricerca svolta sulle biocenosi del macrobenthos nella laguna di Varano e sui contenuti intestinali del *Penaeus japonicus* e *Venerupis semidecussata*. Giugno 1985–Aprile 1986. Università degli Studi di Bari, relazione interna: 23. Piscitelli, G. L'alimentazione naturale di *Penaeus japonicus* Bate: sperimentazione 1982–1985. *Ambiente & Risorse*. 1986;65–70.
12. Galil BS. Truth and consequences: The bioinvasion of the Mediterranean Sea. *Integr Zool*. 2012;7(3):299–311.
13. Galil BS and Zenetos A. A sea change: exotics in the eastern Mediterranean. In: Leppäkoski E & Olenin S (Eds.), *Invasive Aquatic Species of Europe: Distributions, Impacts and Management*. Kluwer Academic Publishers, Netherlands. 2002;325–336.

14. Gilberto R, Héctor S Anthropogenic dispersal of decapod crustaceans in aquatic environments. *Interciencia*. 2001;26:282–288.
15. Kevrekidis K, Kevrekidis T The occurrence of *Penaeus japonicus* in the Aegean Sea. *Crustaceana*. 1996;69(7):925–929.
16. Piscitelli G, Scalera Liaci L Dietanaturale di *Penaeus japonicus* Bate introdotto in estensivo in ambientivallivi e lagunaridella costa adriatica. *Oebalia XI*. 1985;(3) NS 913–915.
17. Gonzalez ML, Gomez Jimenez S, Perez Velazquez M, et al. Nitrogen budget for a low salinity, zero–water exchange culture system: I. Effect of dietary protein level on the performance of *Litopenaeus vannamei* (Boone). *Aquaculture Research*. 2007;38(8):798–808.
18. Madoni P Benthic ciliates in Adriatic sea lagoons. *European Journal of protistology*. 2006;42(3):165–173.
19. Focardi S, Falciai L, Gambi MC, et al. nel Mar Tirreno. XI Congresso SIBM Orbetello. *Atti Soc Tosc Sci Nat Mem ser B*. 1979;86:392–395.
20. Bonilla Gomez JL, Chiappa–Carrara X, Galindo C, et al. Effect of adaptation to the laboratory conditions on growth, molting, and food consumption of juvenile *Farfantepenaeus duorarum* (Decapoda: Penaeidae). *Journal of Crustacean Biology*. 2013;33(2):191–197.
21. Rothlisberg PC Aspects of penaeid biology and ecology of relevance to aquaculture: a review. *Aquaculture*. 1998;164(1–4):49–65.
22. Garcia–Guerrero M, Hernandez–Sandoval P, Orduna–Rojas et al. Effect of temperature on weight increase, survival, and thermal preference of juvenile redclaw crayfish *Cherax quadricarinatus*. *Hidrobiologica*. 2013;23(1):73–81.
23. Du SB, Hu CQ, Shen Q Replacement of a natural diet by a prepared dry feed for successful maturation and spawning of female *Litopenaeus vannamei* (Boone) broodstock. *J World Aquaculture Soc*. 2004;35(4):518–522.
24. Arapi D, Sadikaj R, Malollari I, Ecosystem of cultivation ponds of sewa shrimp (*Marsupenaeus japonicus*) and utilization of its natural food components. *Journal of environmental protection and ecology*. 2012;13(1):375–381.
25. Nguyen BT, Koshio S, Sakiyama K, et al. Effects of polychaete extracts on reproductive performance of kuruma shrimp, *Penaeus japonicus* Bate. – Part II. Ovarian maturation and tissue lipid compositions. *Aquaculture*. 2012;365–67(8):334–337.
26. Bulbul M, Koshio S, Ishikawa M, et al. Performance of kuruma shrimp, *Penaeus japonicus* fed diets replacing fishmeal with a combination of plant protein meals. *Aquaculture*. 2013;45:372–375
27. Bulbul M, Kader, MA, Asaduzzaman M, et al. Can canola meal and soybean meal be used as major dietary protein sources for kuruma shrimp, *Marsupenaeus japonicus*? *Aquaculture*. 2016;452:194–199.
28. Lumare F, Andreoli C, Belmonte G, et al. Growing studies on *Penaeus japonicus* (Decapoda Natantia) in management and environmental diversified conditions. *Riv Ital Piscic IttiopXXI*. 1986;(2):42–58.