Gauchergasilus euripedesi (Copepoda, Ergasilidae) parasitizing different species of fish from two environments in southern Brazil

Moisés Gallas¹, Laura R. P. Utz¹

¹ Laboratório de Ecologia Aquática, Escola de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul. Avenida Ipiranga, 6681, Partenon, Porto Alegre, 90619-900, Rio Grande do Sul, Brazil

Corresponding author: Moisés Gallas (mgallas88@gmail.com)

Abstract
The parasitic copepod Gauchergasilus euripedesi (Montú, 1980) Montú & Boxshall, 2002 was described from plankton samples and specimens found in four fish species from the estuarine area of Patos Lagoon, state of Rio Grande do Sul (RS), Brazil. Later, one different fish species was reported parasitized with G. euripedesi in the same locality. Species of Astyanax Baird & Girard, 1854 (Astyanax henseli Melo & Buckup, 2006 and Astyanax lacustris (Lütken, 1875)) and Psalidodon Eigenmann, 1911 (Psalidodon eigenmanniorum (Cope, 1894) and Psalidodon aff. fasciatus (Cuvier, 1819)) were collected in two environments (Pintada Island, municipality of Porto Alegre and Itapeva Lagoon, municipality of Terra de Areia, RS) to investigate their parasites. The copepods found in the gill arches were counted, processed, mounted in permanent slides, and photographed using light microscopy, or processed for observation in scanning electron microscopy. Specimens of P. eigenmanniorum from Pintada Island, A. lacustris and P. aff. fasciatus from Itapeva Lagoon, were parasitized by G. euripedesi, with prevalences of 29.03% (A. lacustris), 10.34% (P. eigenmanniorum), and 9.68% (P. aff. fasciatus). Measurements obtained for specimens of G. euripedesi were similar to those found in the literature, except for egg sacs which were larger in the specimens examined in the present study. In addition to being the first report of G. euripedesi parasitizing species of fish (A. lacustris, P. eigenmanniorum, and P. aff. fasciatus), the results presented here also extend the known geographic distribution of the copepod species.
Keywords
Astyanax, characid, copepod, ergasilid, Itapeva Lagoon, Pintada Island, Psalidodon

Introduction

The copepod genus Gauchergasilus Montú & Boxshall, 2002 was proposed to accommodate the species Gauchergasilus euripedesi (Montú, 1980) Montú & Boxshall, 2002, anteriorly included in the genus Ergasilus von Nordmann, 1832 (Montú 1980; Montú and Boxshall 2002). In the original description of G. euripedesi, the specimens were collected from plankton samples and from larvae of the following estuarine and marine fish species: Brevoortia pectinata (Jenyns, 1842), Gobiesox sp., Lycengraulis grossidens (Agassiz, 1829), and Micropogonias furnieri (Desmarest, 1823) in the estuarine area of the Patos Lagoon (Montú 1980). Later, redescription of females and corrected description of males, were based on plankton samples collected in the estuarine area of Patos Lagoon (Montú and Boxshall 2002). In addition, females of G. euripedesi were found in M. furnieri by Velloso and Pereira Jr. (2010) in the pre-limnic region of the Patos Lagoon estuary, and in Geophagus brasiliensis (Quoy & Gaimard, 1824) by Rassier et al. (2015) also in the estuarine area of Patos Lagoon. Araujo and Boxshall (2001) reported G. euripedesi obtained from plankton samples collected in the Piauí River estuary. Currently the genus Gauchergasilus is monotypic (Montú and Boxshall 2002; Luque et al. 2013).

The Patos Lagoon system (which includes the Guaíba Lake) comprises the largest lacunar system found in South America (Noronha 1998), and presents at least 170 fish species (Langeani et al. 2009). In Guaíba Lake and its north limit known as Jacuí Delta, species of Astyanax Baird & Girard, 1854 (Astyanax henseli Melo & Buckup, 2006 and Astyanax lacustris (Lütken, 1875)) and Psalidodon Eigenmann, 1911 (Psalidodon eigenmanniorum (Cope, 1894) and Psalidodon aff. fasciatus (Cuvier, 1819)) have been reported (Buckup et al. 2007; Lucena et al. 2013; Terán et al. 2020). From these species, A. lacustris, P. eigenmanniorum, and P. aff. fasciatus occur also in the Tramandaí basin, which includes several interconnected lagoons (Lucena et al. 2013; Malabarba et al. 2013).

In Brazil, parasitic crustaceans have been reported in species of Astyanax and Psalidodon: Argulus japonaramensis Lemos de Castro, 1950, Dolops sp., Ergasilus sp., Paracymothoa astyanaxi Lemos de Castro, 1955, and Lernaea cyprinacea Linnaeus, 1758 in Astyanax bimaculatus (Linnaeus, 1758) (Eiras et al. 2010; Luque et al. 2013; Vasconcelos et al. 2013); Dipteropeltis hirundo Calman, 1912 and Ergasilus sp. in Psalidodon fasciatus (Cuvier, 1819) (Eiras et al. 2010; Luque et al. 2013); Acusicola sp., Amplexibranchii bryconis Thatcher & Paredes, 1985, Brasergasilus sp., Ergasilus sp., L. cyprinacea, and Vaigamus sp. in A. lacustris (Gallio et al. 2007; Eiras et al. 2010; Luque et al. 2013; Pádua et al. 2015; Camargo et al. 2016; Corrêa et al. 2016). Thus, the goal of the present study is to report new hosts and new localities for G. euripedesi in the state of Rio Grande do Sul.
Methods

Specimens of *A. lacustris* (*n* = 42), *A. henseli* (*n* = 35), *P. eigenmanniorum* (*n* = 29), and *P. aff. fasciatus* (*n* = 54) were collected in Pintada Island (30°17’11"S, 51°18’01"W), Jacuí Delta, municipality of Porto Alegre, while specimens of *A. lacustris* and *P. aff. fasciatus* (*n* = 31 each) were sampled from Itapeva Lagoon (29°36’16"S, 49°59’28"W), municipality of Terra de Areia, state of Rio Grande do Sul, southernmost Brazil (Fig. 1). Fish were collected with seine and fyke nets, and packed individually in plastic bags to avoid parasite loss and contact between hosts until necropsy. Host identification followed Bertaco and Lucena (2010), Lucena et al. (2013) and Lucena and Soares (2016). Host systematics followed Terán et al. (2020).

Gills arches of the fishes were removed with the aid of fine-tip scissors, placed in a jar with formalin solution 1:4,000, and shaken at least 50 times (Gallas et al. 2016) to obtain detached copepods. The material was examined under stereomicroscope and the copepods were collected and stored in 70° GL ethanol (Amato et al. 1991). Copepods were mounted *in toto* in Faure’s mounting medium without clarification. Two specimens were prepared for scanning electron microscopy (SEM), where they...
were dehydrated in an ethanol series to critical point dried. The copepods were mounted on stubs, coated with carbon and gold, and then examined in a Field Emission Electron Microscope (FESEM), Inspect F50, FEI at ‘Laboratório Central de Microscopia e Microanálise’ (LabCEMM) at Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil.

Measurements are shown in micrometers (µm) unless otherwise indicated. They represent the minimum and maximum values followed by mean, standard deviation, and sample size in parenthesis. In the description, the terminology follows Huys and Boxshall (1991). Photomicrographs were made using an Olympus BX50 microscope and prepared using ADOBE’S PHOTOSHOP CS2. The parameters of infestations such as prevalence, mean intensity and abundance were used according to Bush et al. (1997). Voucher copepod specimen was deposited in the ‘Coleção de Crustáceos do Museu de Ciências e Tecnologia da PUCRS’ (MCP), Porto Alegre, RS, Brazil.

**Results**

*Gauchergasilus Montú & Boxshall, 2002*

*Gauchergasilus euripedesi* (Montú, 1980) Montú & Boxshall, 2002

Figs 2, 3

Description based on eight adult females. Body cyclopiform, 0.62–0.81 mm (0.71 ± 0.07 mm; \( n = 8 \)) long, disregarding the antennae and the caudal setae. Prosome apparently 5-segmented, 0.45–0.67 mm (0.56 ± 0.07 mm; \( n = 8 \)) long, 200–310 (243 ± 37; \( n = 8 \)) widest in the first segment. Urosome 5-segmented, with small fifth somite; a genital double-somite, and 3-segmented abdomen. Urosome 120–180 (147 ± 22; \( n = 8 \)) long. Genital double-somite 70–90 (76 ± 7; \( n = 8 \)) long, 80–110 (99 ± 11; \( n = 8 \)) wide. Abdomen 50–100 (64 ± 18; \( n = 8 \)) long, 60–70 (62 ± 4; \( n = 8 \)) wide. Caudal rami longer than wide, 20–30 (23 ± 4; \( n = 8 \)) long. Each caudal rami armed with 4 setae, the lateral are longer than the median. Egg sacs 350–780 (557 ± 140; \( n = 6 \)) long, 100–150 (119 ± 21; \( n = 6 \)) wide, fixed in the genital double-somite. Pigments in the body distributed from prosome to abdomen and caudal rami.

Antennules 5-segmented, segmental setation: 11, 5, 4, 2 + aesthetasc, 7 + aesthetasc; antennules 80–95 (87 ± 7; \( n = 5 \)) long. Antennae 4-segmented; coxobasis short and unarmed; first endopodal segment robust, with a seta on inner margin; second endopodal segment curved, with two setae on inner margin, one next to the limit with the first endopodal segment and the other next to the median region; third endopodal segment short; distal claw curved, with a conspicuous barb in the middle of the concave margin. Antennae 330–410 (374 ± 26; \( n = 7 \)) long; distal claw 70–90 (81 ± 8; \( n = 7 \)) long.

Swimming legs 1–4 biramous where all rami are 3-segmented, except 2-segmented endopod in leg 1, and 2-segmented exopod in leg 4. Spine and seta formula of legs 1–4 distributed as follows: all coxae unarmed; all bases with 1 seta on
Figure 2. Photomicrographs of *Gauchergasilus euripedesi*. A. Anterior end, lateral view, showing the body with pigments, antennule (al), antennae (at) and distal claw (white asterisks). B. Anterior end, lateral view, showing the antennae and the third endopodal segment (black arrow heads). C. Prosome and urosome, dorsal view, showing the pigmentation along the body, the swimming legs (sl), genital double-somite (g) and the egg sac (black asterisk). D. Posterior end, showing the genital double-somite (white asterisk) and egg sacs (es). Scale bars: 100 µm (A, B, C); 150 µm (D).

each leg; exopod – leg 1: I-0, 0-1, II,5; leg 2: I-0, 0-1, I,6; leg 3: I-0, 0-1, I,6; leg 4: 0-0, I,5; endopod – leg 1: 0-1, II,5; leg 2: 0-1, 0-2, I,4; leg 3: 0-1, 0-2, I,4; leg 4: 0-1, 0-2, I,3.

Taxonomic summary and parameters of infestations:
Hosts: *Astyanax lacustris* (Lütken, 1875), *Psalidodon eigenmanniorum* (Cope, 1894), and *Psalidodon aff. fasciatus* (Cuvier, 1819).

Localities: Jacuí Delta, Pintada Island, municipality of Porto Alegre, and Itapeva Lagoon, municipality of Terra de Areia, state of Rio Grande do Sul, Brazil.

Site of infestations: gills.

Prevalences: 29.03% (*A. lacustris* from Itapeva Lagoon), 10.34% (*P. eigenmanniorum*), and 9.68% (*P. aff. fasciatus* from Itapeva Lagoon).

Mean intensities of infestations: 1.11 (*A. lacustris* from Itapeva Lagoon), 1 (*P. eigenmanniorum*), and 3.67 (*P. aff. fasciatus* from Itapeva Lagoon).

Mean abundances of infestations: 0.32 (*A. lacustris* from Itapeva Lagoon), 0.1 (*P. eigenmanniorum*), and 0.35 (*P. aff. fasciatus* from Itapeva Lagoon).

Amplitude of intensity of infestations: 1–2 copepods (*A. lacustris* from Itapeva Lagoon), 1 copepod (*P. eigenmanniorum*), and 1–9 copepods (*P. aff. fasciatus* from Itapeva Lagoon).

Voucher specimen of copepod deposited: MCP 3219.

**Figure 3.** Scanning electron microscopy of *Gauchergasilus euripedesi*, dorsal view, showing the antennae (white arrow heads), antennules (red asterisks), genital double-somite (white asterisk) and egg sacs (yellow arrow heads). Scale bar: 150 µm.
Discussion

The ergasilid copepods are described mainly based on their morphology including shape and number of segments in each appendage, segmental setation, in addition to the patterns of spines and setae (Montú 1980; Araujo and Boxshall 2001; Montú and Boxshall 2002). The specimens of *G. euripedesi* found in this study presented all of the traits described by Montú (1980) and Montú and Boxshall (2002) as well as presented the measurements similar to those specimens reported by the two studies. However, the specimens found in the present study presented larger egg sacs (350–780 long) than the specimens (232 long) examined by Montú (1980). These differences could be the result of incompletely developed egg sacs of the specimens analyzed by Montú (1980) or due to a variation not reported until now. Therefore, most measurements in the present study represent new data for *G. euripedesi*.

The parameters of infestations of *G. euripedesi* available are presented in Table 1. In general, parameters of the host species examined in the present study are more similar to those reported for *M. furnieri* (Velloso and Pereira Jr. 2010), but smaller than those found in *G. brasiliensis* (Rassier et al. 2015). These differences do not seem to be related to the number of hosts examined, since the two most sampled hosts (*M. furnieri*, *n* = 94 and *G. brasiliensis*, *n* = 79) presented differences between them. Moreover, they also presented differences in comparison with the hosts examined in the present study (*A. lacustris*, *n* = 31; *P. eigenmanniorum*, *n* = 29; and *P. aff. fasciatus*, *n* = 31). In addition, considering the tolerance to salinity variations, *G. euripedesi* prefers low salinity levels found in the Patos Lagoon estuary (Velloso and Pereira Jr. 2010), thus, the infestations found in Guaíba Lake and Itapeva Lagoon should be higher than those reported by Velloso and Pereira Jr. (2010) and Rassier et al. (2015). More studies are necessary to explain the different levels of infestations of *G. euripedesi*. Other factors such as the availability of hosts, presence of different hosts species in the same area, characteristics of the environments, to name a few, must be considered since salinity levels do not seem to be the only or the main factor influencing infestations by *G. euripedesi*.

In Brazil, the ergalisid crustaceans that have been reported in *A. lacustris* and *P. fasciatus* include one species (*A. bryconis*) and four undetermined species (*Acusicola* sp., *Brasergasilus* sp., *Ergasilus* sp., and *Vaigamus* sp.) (Eiras et al. 2010; Luque et al. 2013; Pádua et al. 2015; Camargo et al. 2016). Until now, previous reports of fish parasitized by *G. euripedesi* include larvae of *B. pectinata*, *Gobiesox* sp., *L. grossidens*,

Table 1. Comparison of the parameters of infestations of *Gauchergasilus euripedesi* in different studies.

| Host                          | Prevalence (%) | Mean intensity | Mean abundance | Amplitude of intensity | Reference |
|-------------------------------|----------------|----------------|----------------|------------------------|-----------|
| *Micropogonias furnieri* (*n* = 94) | 14.89          | 2.79           | 0.41           | *                      | Velloso and Pereira Jr. (2010) |
| *Geophagus brasiliensis* (*n* = 79) | 32.91          | 2.42           | 0.79           | 1‒63                   | Rassier et al. (2015) |
| *Astyanax lacustris* (*n* = 31) | 29.03          | 1.11           | 0.32           | 1‒2                    | Present study |
| *Psalidodon eigenmanniorum* (*n* = 29) | 10.34          | 1              | 0.1            | 1                      | Present study |
| *Psalidodon aff. fasciatus* (*n* = 31) | 9.68           | 3.67           | 0.35           | 1‒9                    | Present study |

* not given by the authors.
Moisés Gallas & Laura R.P. Utz

and *M. furnieri* (Montú 1980), and adult specimens of *G. brasiiliensis* and *M. furnieri* (Velloso and Pereira Jr. 2010; Rassier et al. 2015). In additional reports, the specimens of *G. euripedesi* were collected from plankton samples, since only females are known to be parasitic (Araujo and Boxshall 2001; Montú and Boxshall 2002). This is the first report of *G. euripedesi* parasitizing species of *Astyanax* (*A. lacustris*) and *Psalidodon* (*P. eigenmanniorum* and *P. aff. fasciatus*), in which all species are considered new hosts. These new reports reflect the potential high number of parasitic crustaceans that are still underestimated as pointed out by Luque et al. (2013).

This study also extends the known geographic record of *G. euripedesi* in the lagoon system of Patos Lagoon to the Jacuí Delta, and includes a new locality record (Itapeva Lagoon). It is possible that *G. euripedesi* could be found in other localities throughout the territory between southern and northeast Brazil since its distribution is only known in the states of Rio Grande do Sul and Sergipe (Araujo and Boxshall 2001; Montú and Boxshall 2002).

**Conclusions**

The specimens of *G. euripedesi* presented morphology and measurements (except for large egg sacs reported here) similar to the specimens reported in previous studies. The species *A. lacustris*, *P. eigenmanniorum*, and *P. aff. fasciatus* are reported as new hosts for *G. euripedesi*, thus, increasing the list of fishes species parasitized by crustaceans in Brazil. The present study also extends the known distribution of *G. euripedesi* in southern Brazil and contributes to the knowledge of the biodiversity of copepod parasites in the region.

**Acknowledgements**

We thank fishermen for host collection and field assistance; to Dr Eliane F da Silveira for help with host collection and transportation; to Bucal Pathology Laboratory (Faculty of Odontology), PUCRS for permission to use the microscope to take the photomicrographs; to ‘Laboratório Central de Microscopia e Microanálise’ (LabCEMM) at PUCRS for the procedures and photomicrographs of SEM; MG also thanks the Brazilian National Research Council (CNPq) for financial support (process #140639/2016-0).

**References**

Amato JFR, Boeger WA, Amato SB (1991) Protocolos para laboratório – coleta e processamento de parasitos do pescado. Imprensa Universitária da Universidade Federal Rural do Rio de Janeiro, Seropédica, 81 pp.

Araujo HMP, Boxshall GA (2001) A new species of Acusicola Cressey (Copepoda: Ergasilidae) from northeastern Brazil. Systematic Parasitology 49(2): 149–157. https://doi.org/10.1023/A:1010624822047
Gauchergasilus euripedesi parasitizing species of fish in southern Brazil

Bertaco VA, Lucena CAS (2010) Redescription of Astyanax obscurus (Hensel, 1870) and A. laticeps (Cope, 1894) (Teleostei: Characidae): two valid freshwater species originally described from rivers of Southern Brazil. Neotropical Ichthyology 8(1): 7–20. https://doi.org/10.1590/S1679-62252010000100002

Buckup PA, Menezes NA, Ghazzi MS [Eds] (2007) Catálogo das Espécies de Peixes de Água Doce do Brasil. Museu Nacional, Rio de Janeiro, 195 pp.

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. The Journal of Parasitology 83(4): 575–583. https://doi.org/10.2307/3284227

Camargo AA, Negrelli DC, Pedro NHO, Azevedo RK, Silva RJ, Abdallah VD (2016) Metazoan parasite of lambari Astyanax altiparanae, collected from the Peixe river, São Paulo, southeast of Brazil. Ciência Rural 46(5): 876–880. https://doi.org/10.1590/0103-8478cr20151100

Corrêa LL, Tavares-Dias M, Ceccarelli PS, Adrianio EA (2016) Hematological alterations in Astyanax altiparanae (Characidae) caused by Lernaea cyprinacea (Copepoda: Lernaeidae). Diseases of Aquatic Organisms 120(1): 77–81. https://doi.org/10.3354/dao03008

Eiras JC, Takemoto RM, Pavanelli GC (2010) Diversidade dos parasitas de peixes de água doce do Brasil. Clichetec, Maringá, 333 pp.

Gallas M, Calegaro-Marques C, Amato SB (2016) A new species of Characithecium (Monogenea: Dactylogyridae) from external surface and gills of two species of Astyanax (Ostariophysi: Characidae) in southern Brazil. Revista Mexicana de Biodiversidad 87(3): 903–907. https://doi.org/10.1016/j.rmb.2016.06.011

Gallio M, Silva AS, Monteiro SG (2007) Parasitismo por Lernaea cyprinacea em Astyanax bimaculatus provenientes de um acuade no município de Antonio Prado, Rio Grande do Sul. Acta Scientiae Veterinariae 35(2): 209–212. https://doi.org/10.22456/1679-9216.15973

Huys R, Boxshall GA (1991) Copepod Evolution. The Ray Society, London, 468 pp.

Langeani F, Buckup PA, Malabarba LR, Py-Daniel LHR, Lucena CAS, Rosa RS, Zuanon JAS, Lucena ZMS, Brito MR, Oyakawa OT, Gomes-Filho G (2009) Peixes de água doce. In: Rocha RM, Boeger WA (Orgs) Estado da Arte e Perspectivas para a Zoologia no Brasil. Editora UFPR, Curitiba, 211–230.

Lucena CAS, Soares HG (2016) Review of species of the Astyanax bimaculatus “caudal peduncle spot” subgroup sensu Garutti & Langeani (Characiformes, Characidae) from the rio La Plata and rio São Francisco drainages and coastal systems of southern Brazil and Uruguay. Zootaxa 4072: 101–125. https://doi.org/10.11646/zootaxa.4072.1.5

Lucena CAS, Castro JB, Bertaco VA (2013) Three new species of Astyanax from drainages of southern Brazil (Characiformes: Characidae). Neotropical Ichthyology 11(3): 537–552. https://doi.org/10.1590/S1679-62252013000300007

Luque JL, Vieira FM, Takemoto RM, Pavanelli GC, Eiras JC (2013) Checklist of Crustacea parasitizing fishes from Brazil. Check List 9(6): 1449–1470. https://doi.org/10.15560/9.6.1449

Malabarba LR, Neto PC, Bertaco VA, Carvalho TP, Santos JR, Artioli LGS (2013) Guia de identificação dos peixes da Bacia do Rio Tramandaí. Editora Via Sapiens, Porto Alegre, 140 pp.
Montú MA (1980) Parasite copepods of Southern Brazilian Fishes. I. *Ergasilus euripedesi* n. sp. (Copepoda, Cyclopoidea). Iheringia. Série Zoologia 56: 53–62.

Montú MA, Boxshall GA (2002) *Gauchergasilus*, a new genus for *Ergasilus euripedesi* Montú, 1980, an abundant parasitic copepod from the Patos Lagoon in southern Brazil. Systematic Parasitology 51(1): 21–28. https://doi.org/10.1023/A:1012985717903

Noronha LC [Ed.] (1998) Baía de todas as águas: preservação e gerenciamento ambiental na Bacia Hidrográfica do Guaíba. Secretaria da Coordenação e Planejamento do Estado do Rio Grande do Sul, Porto Alegre, 112 pp.

Pádua SB, Jerônimo GT, Menezes-Filho RN, Taboga SR, Martins ML, Belo MAA (2015) Pathological assessment of farmed yellowtail tetra *Astyanax altiparanae* infested by *Acusicola* sp. (Ergasilidae). Aquaculture Reports 2: 63–66. https://doi.org/10.1016/j.aqrep.2015.08.003

Rassier GL, Pesenti TC, Pereira Júnior J, Silva DS, Wendt EW, Monteiro CM, Berne MEA (2015) Metazoan parasites of *Geophagus brasiliensis* (Perciformes: Cichlidae) in Patos lagoon, extreme south of Brazil. Revista Brasileira de Parasitologia Veterinária 24(4): 447–453. https://doi.org/10.1590/S1984-29612015075

Terán GE, Benítez MF, Mirande JM (2020) Opening the Trojan horse: phylogeny of *Astyanax*, two new genera and resurrection of *Psalidodon* (Teleostei: Characidae). Zoological Journal of the Linnean Society 190(4): 1217–1234. https://doi.org/10.1093/zoolinnean/zlaa019

Vasconcelos ACP, Lopes ACM, Santos JMS, Jeraldo VLS, Melo CM, Madi RR (2013) Molecular analysis and biodiversity of metazoan parasites of the yellow tail lambari, *Astyanax aff. bimaculatus* (Teleostei, Characidae), in lower San Francisco, northeastern Brazil. Neotropical Helminthology 7(1): 41–49.

Velloso AL, Pereira Jr J (2010) Influence of ectoparasitism on the welfare of *Micropogonias furnieri*. Aquaculture (Amsterdam, Netherlands) 310(1): 43–46. https://doi.org/10.1016/j.aquaculture.2010.10.030