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Research Article

Broodstock management of the fine flounder *Paralichthys adspersus* (Steindachner, 1867) using recirculating aquaculture systems

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ABSTRACT. The present study describes the methodology used at IMARPE for the capture, acclimation and management of *P. adspersus* broodstock using recirculating aquaculture systems (RAS). RAS improved the water quality and maintained the environmental parameters during the acclimation period, temperature (17.2±1ºC), oxygen (8.1±0.7 mg L^{-1}), pH (7.3±0.2), ammonia (0.004±0.003 mg L^{-1}), nitrite (0.52±0.2 mg L^{-1}) and nitrate (3.45±2.6 mg L^{-1}). Fish began to be fed normally from day 15 post-capture, once or twice a day using live fish (*Odonthestes regia regia, Mugil cephalus*), crustacean (*Emerita analoga*), fresh food (*Engraulis ringens* and *Dosidicus gigas*) and artificial feed. A significant loss in the weight of the fish was registered during the first days of captivity, followed by a continuous increase in both sexes. The specific growth rate was positive from the third month of captivity, being the relative growth rate 24.5% and 16.2% in August 2010 in males and females, respectively. Different internal and external parasites were detected in the fish, being *Entobdella* sp. and *Philometra* sp. the prevailing parasites observed during samplings.

Keywords: *Paralichthys adspersus*, reproduction, recirculating aquaculture system, broodstock management, culture, Peru.

Manejo de reproductores del lenguado *Paralichthys adspersus* (Steindachner, 1867) usando sistemas de recirculación en acuicultura

RESUMEN. El presente trabajo, describe la metodología desarrollada en IMARPE para la captura, aclimatación y acondicionamiento de ejemplares adultos de *P. adspersus* en sistemas de recirculación (SRA), con la finalidad de formar un stock de reproductores. El SRA permitió manejar parámetros medioambientales estables durante el periodo de acondicionamiento, como: temperatura del agua (17,2±1ºC), oxígeno disuelto (8,1±0,7 mg L^{-1}), pH (7,3±0,2), amonio (0,004±0,003 mg L^{-1}), nitrito (0,52±0,2 mg L^{-1}) y nitrato (3,45±2,6 mg L^{-1}). Se dio inicio a la alimentación el día 15 post-captura, utilizando alimento vivo (*Odonthestes regia regia*, *Mugil cephalus*), crustáceos (*Emerita analoga*), alimento fresco (*Engraulis ringens* y *Dosidicus gigas*) y artificial semihúmedo. Durante los primeros días de acondicionamiento los peces mostraron una disminución en el peso, hasta su adaptación a las condiciones de cultivo, luego de lo cual se produjo un incremento continuo en ambos sexos. La tasa específica de crecimiento fue positiva a partir del tercer mes y la tasa de crecimiento relativo mostró que en agosto 2010, el peso promedio se incrementó 24,5% en machos y 16,2% en hembras. Se realizó un análisis patológico a los ejemplares capturados y se observó la presencia de diferentes parásitos internos y externos, entre ellos predominaron *Entobdella* sp. y *Philometra* sp.

Palabras clave: *Paralichthys adspersus*, reproducción, sistema de recirculación en acuicultura, manejo de reproductores, cultivo, Perú.

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INTRODUCTION

The fine flounder *Paralichthys adspersus* (Steindachner, 1867) is a flatfish with high commercial value in Peru and Chile, and considered a new species for aquaculture taking into account the excellent quality of its filet, the decline of natural populations and the increasing local demand.

Paralichthys adspersus (Steindachner, 1867) is distributed from Paita (Peru) to Lota and Juan Fernández Islands (Chile) (Acuña & Cid, 1995). In the last years the interest in its culture has increased due to the high marketable price and the important national and international demand of the species. However, little knowledge of its biology and aquaculture exists (Silva & Flores, 1989; Chinchayán *et al*., 1997; Silva, 2001; Angeles & Mendo, 2005; Piaget *et al*., 2007) to allow the development of a profitable commercial culture.

Abundant literature exists on broodstock management and culture technology of other flatfishes of the genus *Paralichthys* such as *P. olivaceus* in Japan (Alam *et al*., 2002; Furuita *et al*., 2002; Hernández *et al*., 2007; Yamaguchi *et al*., 2007), *P. microps* in Chile (Silva, 2001), *P. californicus* (Conklin *et al*., 2003; Gisbert *et al*., 2004; Merino *et al*., 2007; Palumbo *et al*., 2007) and *P. lethostigma* (Smith *et al*., 1999; Watanabe & Carroll, 1999) in the United States, *P. orbignyanus* in Argentina and Brazil (Bambill *et al*., 2006; Müller *et al*., 2006; Lanes *et al*., 2009, 2010); although little is known about the use of Recirculating Aquaculture System (RAS) technology for broodstock culture of *P. adspersus*.

RAS technology is very useful for fish production on a commercial scale due to the lower land and water demanded, the increased control over water quality, the maintenance of constant enviromental factors such as temperature, pH, salinity and photoperiod, the reduction of environmental impact and the increase in biosecurity. Thus, the present study was designed to establish the management of *Paralichthys adspersus* broodstock in captivity and the use of RAS for this species.

MATERIALS AND METHODS

Capture and transport of fish

Wild flounder fish were captured by means of cast nets (atarraya) in the north-central area of Lima, between November and December 2009 (Fig. 1). The fish were placed in 0.3 m^3 fiber-glass tanks with constant aeration and temperature (15ºC) using icepacks. In addition, 0.125 mL L^{-1} of sea water conditioner AquaSafe® was added to keep water in good conditions and to reduce the stress of the fish during transportation (6 h) to the Laboratory of Fish Culture of the Instituto del Mar de Perú (IMARPE) at Callao (Peru).

Facilities, RAS and water quality

Two 7 HP water pumps located in the IMARPE pier were used for water supply to RAS (Recirculating Aquaculture System). Water passed through four filters (sand, gravel, quartz and stone, placed in series) and stored in a 50 m^3 reservoir tank (Fig. 2a). Wild flounders were placed in six 2.5 m^3 tanks, connected to 2 RAS with 73 m^2 surface area. Each RAS was composed by three blue colored, self-cleaning, cylindrical tanks, a 1/3 HP water pump, a 4.5 ft^3 floating bed filter (FBF) working as a mechanical and biological filter, a 9000 BTU heat pump for temperature control, a 40 WUV light sterilizer (Fig. 2b), two 20 µm cartridge filters (cuno) placed in parallel and a ½ HP blower to keep oxygen levels. Each tank was covered with a black geomembrane and a 150 W lamp was placed above the water surface and connected to a programmable lighting system to adjust the photoperiod.

Oxygen measured with a 3 Star Oxymeter (Thermo Scientific®), pH measured with a HI-98160 pH meter (Hanna®) and carbon dioxide (CO_2), total ammonia nitrogen (TAN), nitrite (NO_2) and nitrate (NO_3) measured with LaMotte® kits, were recorded daily (early morning) and un-ionized ammonia nitrogen (N-NH_3) calculated using the method by Khoo *et al*. (1977). Temperature was recorded continuously using Tidbit temperature sensors, data loggers HOBO®, registering temperature values every 30 min, the data was downloaded using software HOBO ware Pro 2.7.2.

Additional groups of fish were kept in a 0.3 m^3 circular fiberglass tank for periodic parasitological analysis (PAT).

Food

The fish were starved for 15 days. After this period, the fish were fed once or twice per day (11:00 h and

13:00 h) with juvenile live fish (*Odonthestes regia regia*, *Mugil cephalus*), crustacean (*Emerita analoga*), fresh food (*Engraulis ringens* and *Dosidicus gigas*) and artificial feed. The artificial semi-moist feed was made by mixing fish and giant squid meal, fish oil, fresh anchovy, giant squid and vitamin premix. The fish were fed 0.5% of their body weight (BW) on Monday formulated feed, and on Wednesday and Friday fresh or live fish. Uneaten food was weighed in order to calculate daily food intake and the feeding rate was periodically adjusted according to fish growth.

Biometric sample and deworming

Fish were anesthetized with MS-222 solution (80 mg L^{-1}) to record total length (cm), weight (g) and gonadal maturity every month. Fish were injected monthly a 10% enrofloxacin solution (0.05 mL kg^{-1}) to prevent infections, whereas 3-5 min fresh water baths were used to eliminate external parasites. Recently catch (CAT), parasitological analysis (PAT) and RAS fish were sacrificed to check internal parasites. Different organs were dissected for analysis: brain, gills, heart, spleen, liver, kidney, muscle and skin, according to the methodology described by Millemann (1968).

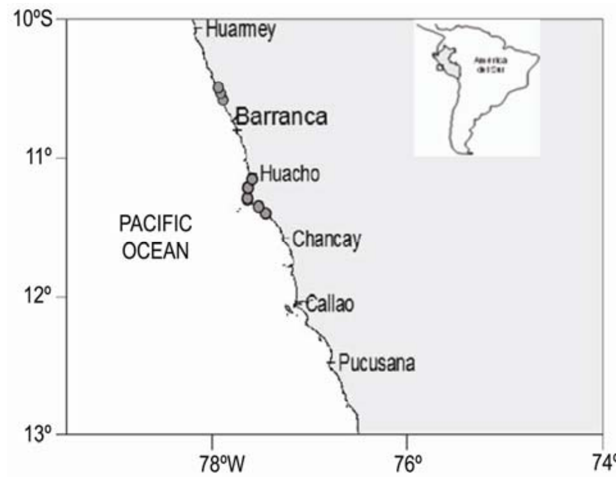

Figure 1. Fishing area of wild flounders used for broodstock in the present study. Circles indicate the sampling stations.

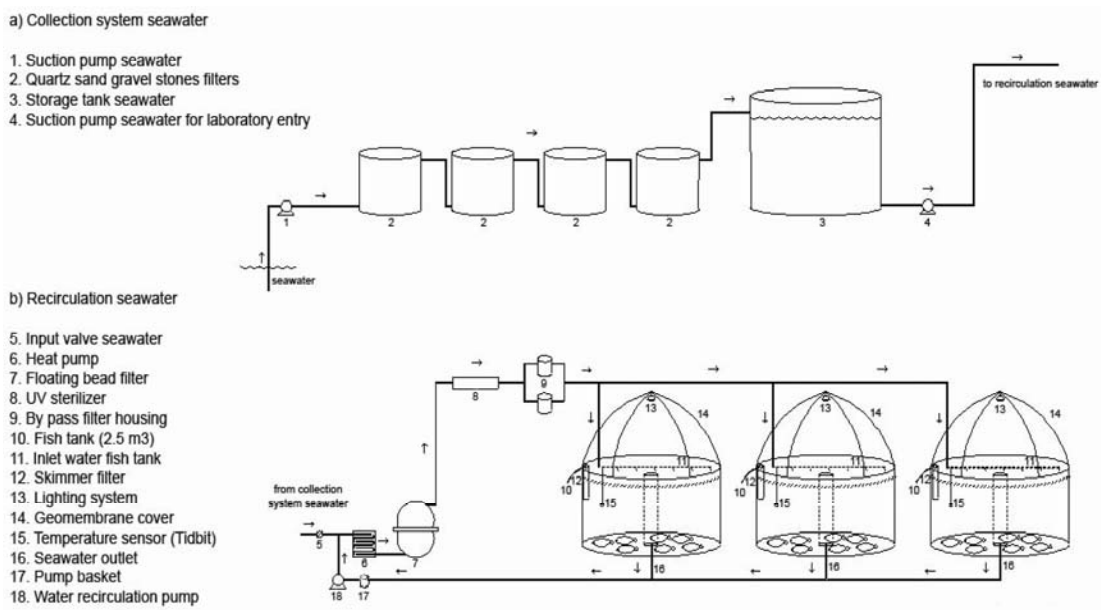

Figure 2. Water supply system (a) and recirculation culture units (b) designs used in the study.

Tagging and sexing

Fish were PIT (Passive Integrated Transporter) tagged on the dorsal muscle for individual identification. The codes of 121 fish were read with an Allflex® reader immediately after being tagged. Besides, two non-invasive techniques, visual inspection of genital opening and cannulation using a 0.8 mm diameter catheter (Watanabe & Carroll, 1999) were used for sex identification.

Biological parameters

Total weight (W_T) of fish was recorded every 45 days and used to calculate specific growth rate (SGR) and relative growth (RG) according to the following formula:

$$SGR\ i = ([(W_T)\ i - (W_T)_{(i\text{-}1)}] \text{ x } [(t_i - t_{(i\text{-}1)})^{(-1)} \text{ x } 100])$$
$$RGi = [(W_T)\ i - (\mathrm{W}_T)_{(i\text{-}1)}] \text{ x } W_T^{(-1)} \text{ x } 100]$$

RESULTS

No mortality was recorded during transport, but 11.4% of the fish died during the acclimation period (cumulative mortality until September 2010). Surviving fish (121) were then distributed in two RAS units at a density of 2-3 kg·m^2 and a sex ratio of 2:1 (female: male). Water flow rate was kept at 10-15 L min^{-1}, with a total water change in the tank every 166-250 min. Water parameters were stable and within the ranges recommended for other species of flatfishes (Poxton *et al.*, 1982; Dinis *et al.*, 1999; Smith *et al.*, 1999; Katersky *et al.*, 2006). Nitrogen levels showed the typical curve for the maturation of biofilters (Timmons *et al.*, 2002) at the beginning but after that NH_3 levels remained below 0.0125 mg L^{-1}. The levels of N-NH_3 and CO_2 in the water only increased when feeding rate increased (Figs. 3a-3b).

PIT tagging and cannulation did not cause any mortality and no loss of tags was recorded. Although flounders do not show external dimorphism between males and females, females have genital opening with an "8" appearance with two pores located on the blind side (Fig. 4a), whereas in the case of males, it is smaller and narrower being located on the ocular side (Fig. 4b).

Fish feeding started with live fish given *ad libitum*, and then fresh fish and formulated feed were added. Feeding rate gradually increased according to consumption and increase in body biomass. Live fish and formulated feed given to the fish increased until a maximum of 2.8 and 2.4% of BW respectively, while fresh fish increased up to 4% of BW.

Formulated semi moist diet showed low stability dropping to the bottom of the tank where it disaggregated rapidly causing water quality problems. Although this problem was solved increasing the amount of the binder (carboxymethyl cellulose) from 0.5 to 2.5%, the diet had low palatability showing the

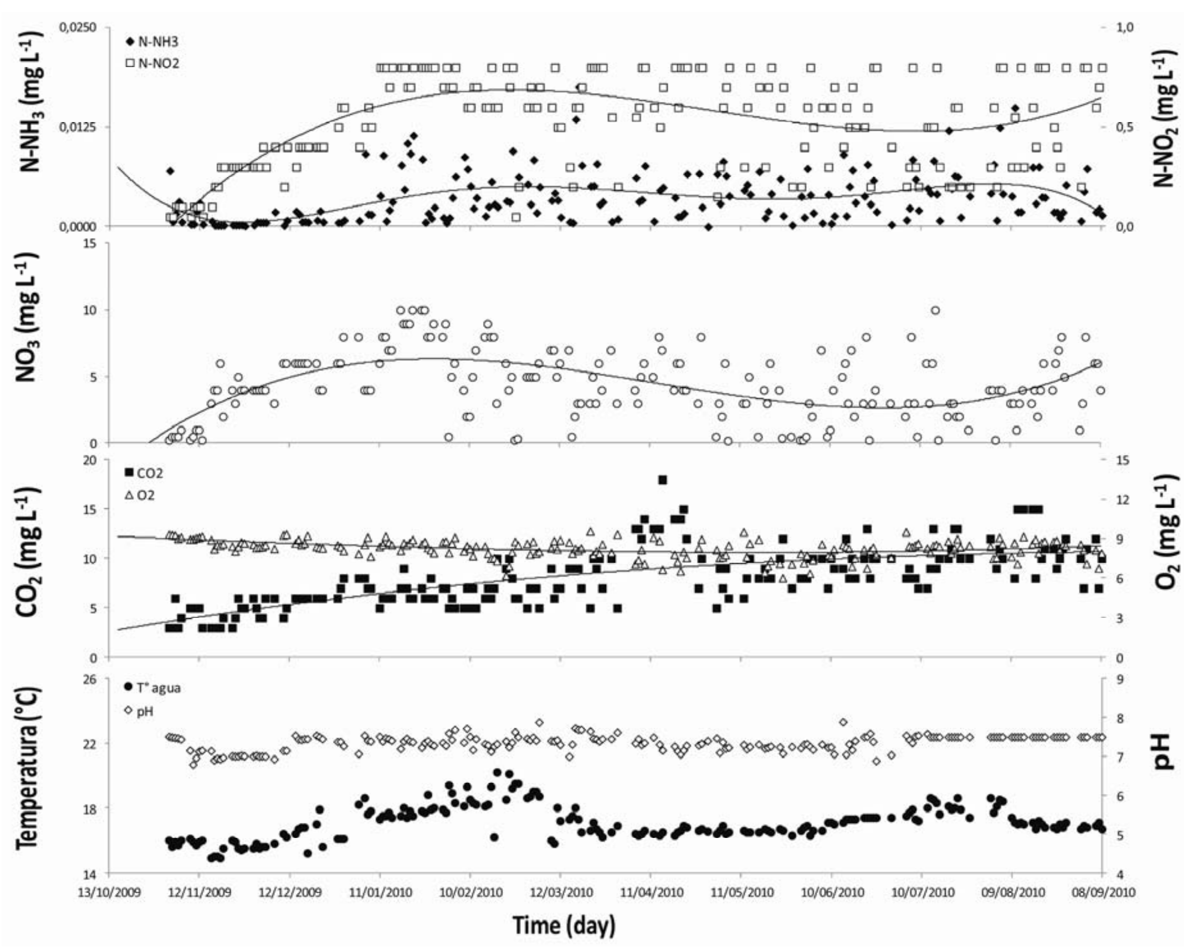

Figure 3a. Water quality parameters of recirculation unit 1.

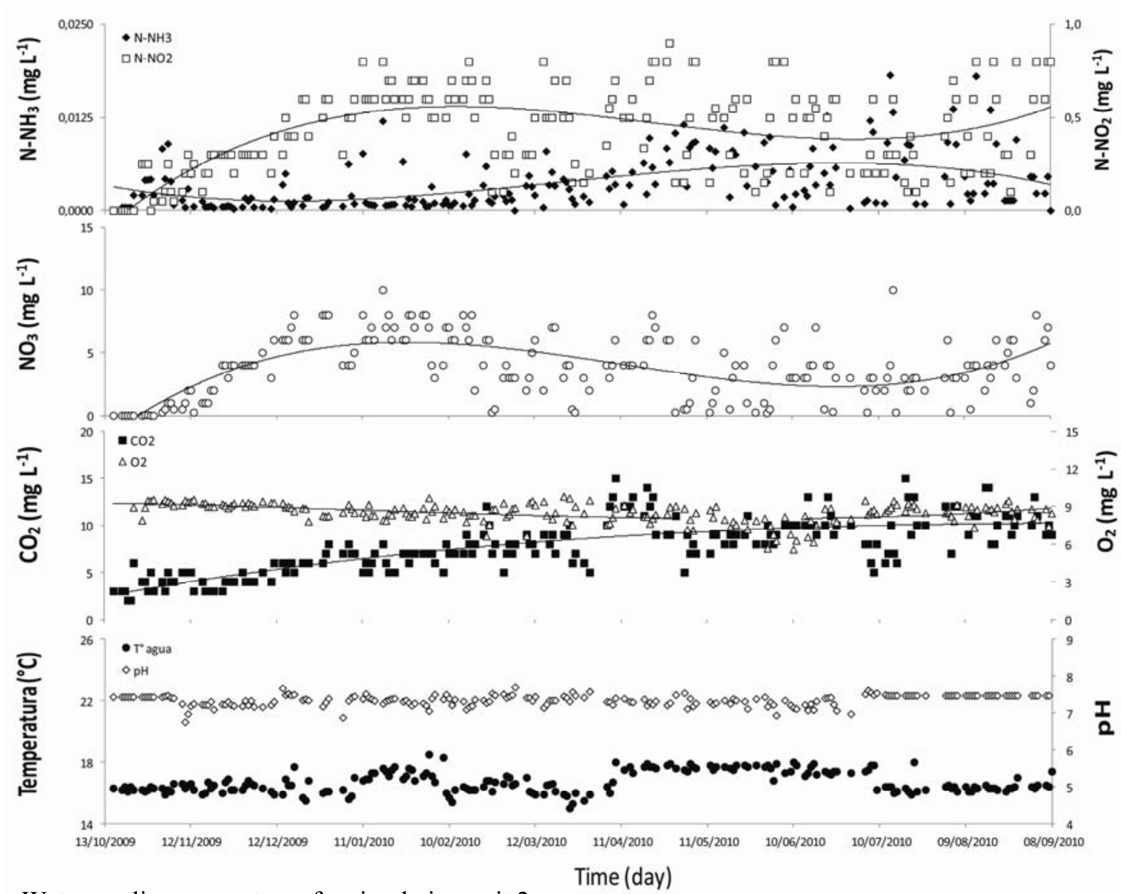

Figure 3b. Water quality parameters of recirculation unit 2.

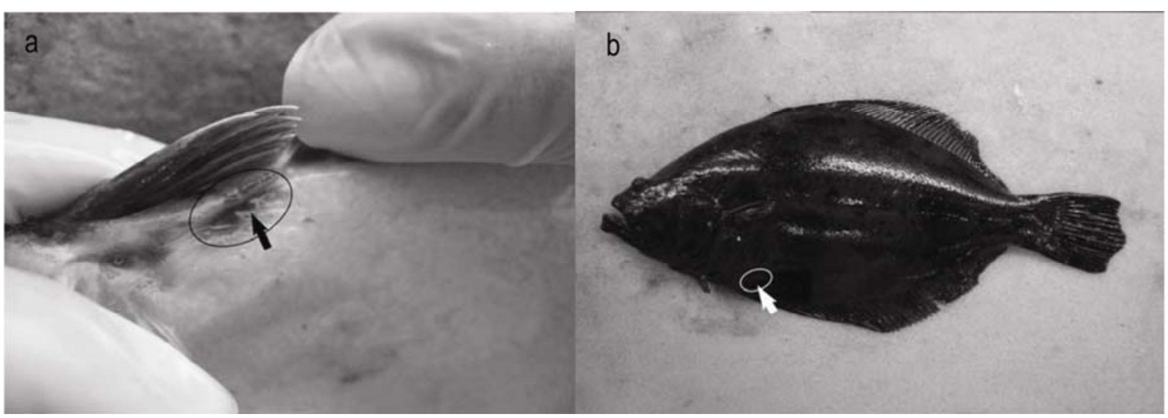

Figure 4. a) Female genital opening, b) male urogenital opening. Circles indicate location of genital opening.

fish a very low ingestion, thus the proportion of this diet was reduced with respect to live fish fed to flounders (Figs. 5a-5b).

The weight recorded for females and males was significantly different, with an initial average weight of 992 and 631 g, respectively. During the first 60 days of acclimation to captivity fish of both sexes lost weight showing a continuous increase afterwards (Fig. 6a). SGR values showed a clear positive increment in both sexes from the third month of captivity whereas in the case of RGR the values increased by August being the average weight 24.5% higher in males and 16.2% higher in females (Figs. 6b-6c).

Different internal and external parasites were observed in broodstock fish along the rearing (Fig. 7). *Philometra* sp. was the predominant internal parasite

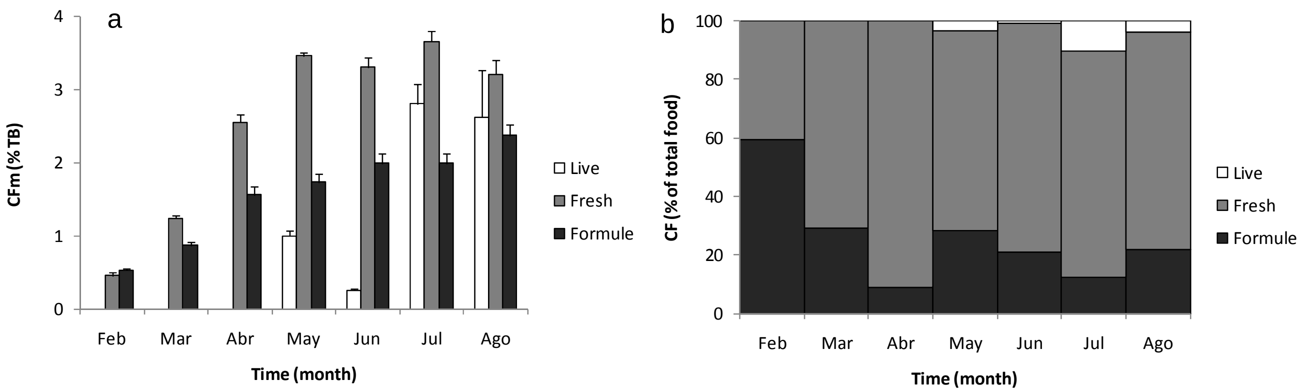

Figure 5. Food consumed by fine flounder (CF). a) Monthly average of food consumption in % total body weight (TB), b) food consumed in % by type of food.

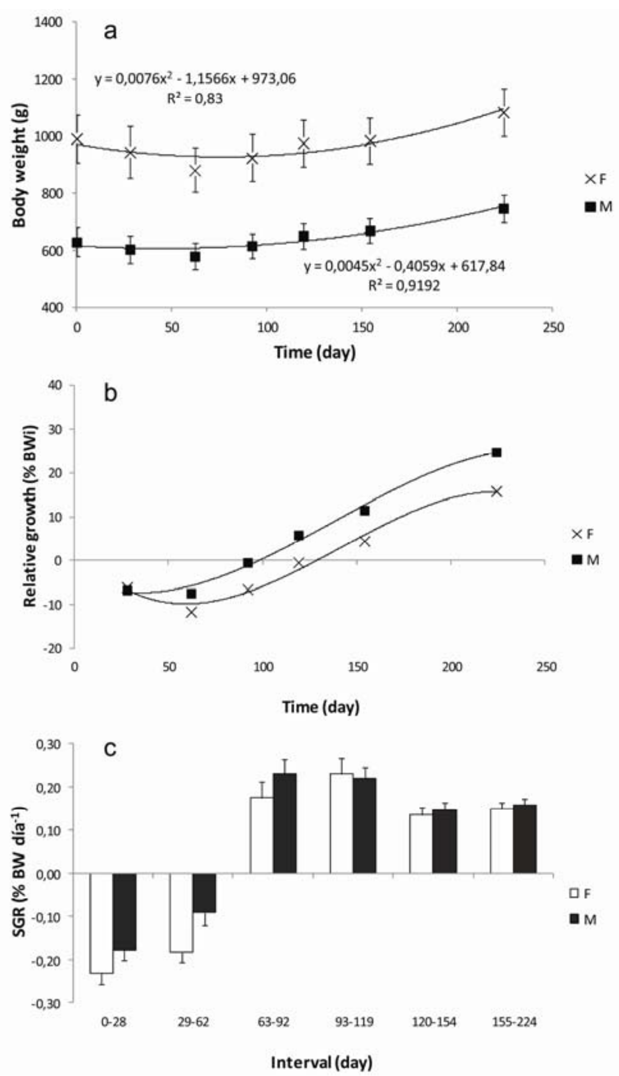

Figure 6. Growth rate of fine flounder reared in recirculation systems. a) Weight gain, b) relative growth rate (RGR), and c) specific growth rate (SGR). Day 0 corresponds to January 2009.

but its proliferation was prevented by installing 20 µm filters in the water inlet to retain the larvae and intermediate hosts. Thus, *Philometra* sp. was eradicated from the system by July (Fig. 8a). *Entobdella* sp. was the most frequent external parasite (Fig. 8b) in this case to control and avoid spreading of this and other external parasites in the RAS, fish were treated weekly with freshwater baths.

DISCUSSION

The survival rate of *P. adspersus* during and after capture was 100%, due to the net used for fishing (atarraya) where the fish were individually caught. No physical damage was detected on the fish thanks to the short contact time (2 to 3 min) of the fish with the net that allowed no scale loss and stress. Silva & Oliva (2010) using another type of fishing gear (drive) got post-capture survival rates between 60 and 80%. In that case fish were captured in groups and physical damage due to overcrowding was observed in the fish.

One of the main bottlenecks of RAS is the elimination of toxic metabolites produced by feces, urine and microbial decomposition of uneaten food that need to be removed from the rearing water by the action of bio filters. All types of biofilters described in literature (Losordo *et al*., 1999) go through a process of maturation to form a colony of nitrifying bacteria that remove and transform ammonia in nitrites and then in nitrates. The biofilter used in the RAS described in the present study went through a maturation period of weeks, working efficiently afterwards and keeping the levels of ammonia in 0.004 mg L^{-1} on average, similarly to the levels obtained by Timmoms *et al.* (2002), Ingle de la Mora *et al*. (2003) and Summerfelt *et al.* (2004) and recommended for

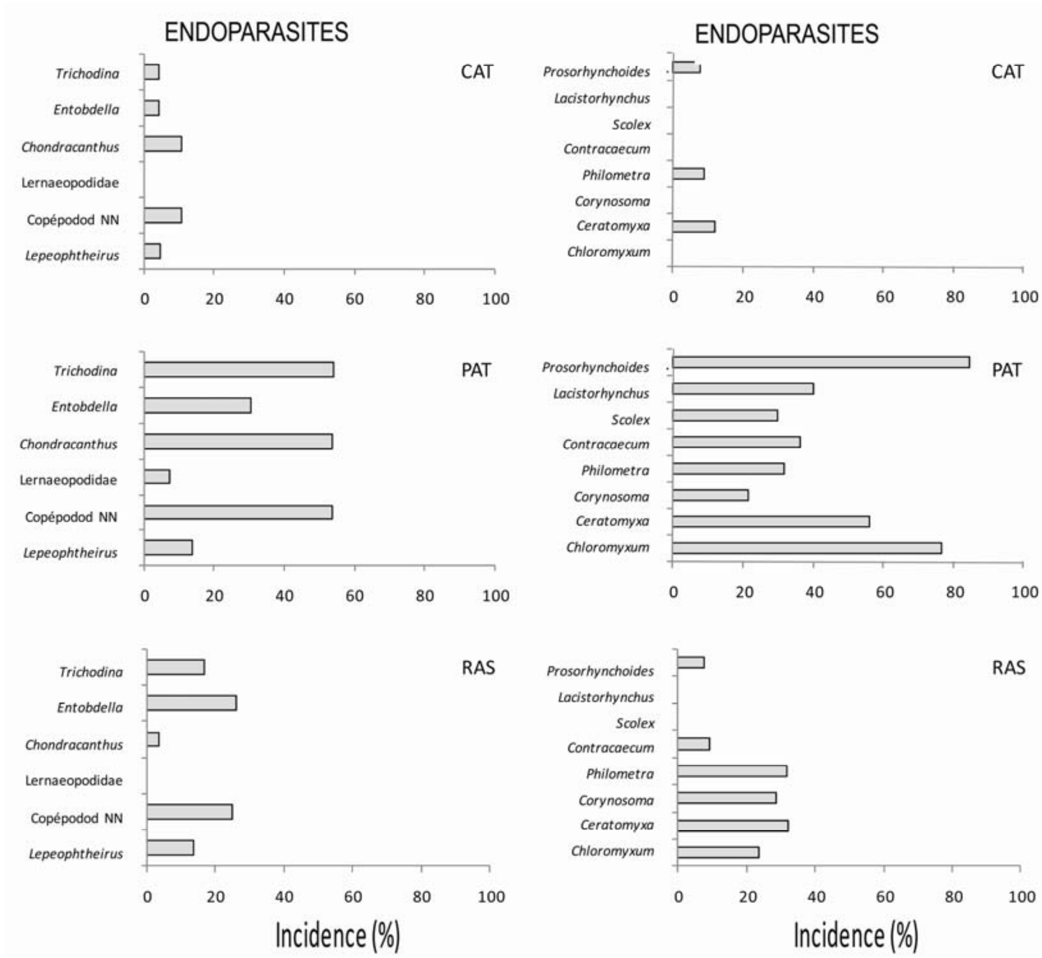

Figure 7. Incidence of ecto and endo parasites in wild fish catched (CAT), fish kept in 300 L tank for parasitological analysis (PAT) and fish kept in recirculation aquaculture systems (RAS).

similar species such as *Scophthalmus maximus* L. (Poxton *et al.*, 1982), *Solea senegalensis* (Dinis *et al.*, 1999), *Paralichthys lethostigma* (Smith *et al.*, 1999), *P. dentatus* (Katersky *et al.*, 2006).

Sexual dimorphism between females and males is very clear for some fish species with differences in body shape, coloration, caudal fin shape, number of genital openings, etc. However, most flatfish do not show any morphological difference between sexes and either cannulation or ovarian biopsy is needed (Vecsei *et al.*, 2003). In this study with *P. adspersus* the identification of females and males was carried out by cannulation.

Successful encoding of fish is considered by Thomassen *et al.* (2000) when low (1) mortality due to handling and (2) loss of tags is recorded. Thus, in the present study, PIT tagging of *P. adspersus* did not cause any fish mortality or loss of tags. This type of marking is used not only for aquaculture purposes but also in conservation programs, sport fishing and genetic improvement programs (Navarro *et al.*, 2004, 2006; Astorga *et al.*, 2005; Carrillo *et al.*, 2009).

In teleost, fish maturation and successful reproduction are closely related to the nutritional status of the fish, thus poor nutrition prior or during the spawning period has clear effects either on the growth of the fish, their sexual maturation, and on the quantity and quality of spawning (Carrillo *et al.*, 2000). In the present study *P. adspersus* feeding process went through several stages until the fish accepted fresh food and formulated diets, in an attempt to cover all their nutritional requirements, for a future evaluation of gonadal maturity and quality of eggs and larvae.

Furthermore, wild captured *P. adspersus* showed a high prevalence of endo and ectoparasites that affected food consumption, produced petechiae and consequently had a clear influence on fish condition. Other authors (Oliva *et al.*, 1996) reported the parasites found in *P. adspersus* and cited mainly *Entob-*

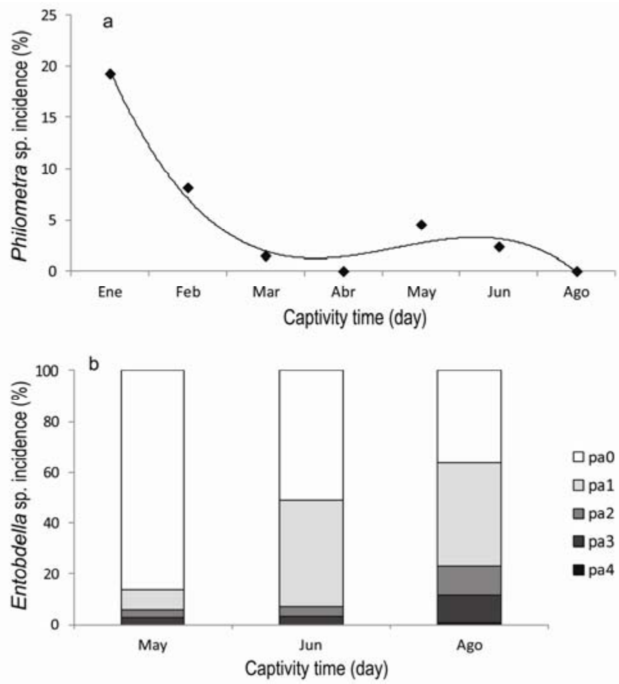

Figure 8. a) Incidence of *Philometra* sp., and b) *Entobdella* sp. in fine flounder reared in water recirculation systems. Day 0 corresponds to January 2009.

della sp. and *Philometra* sp. with higher prevalence in females than in males. Treatments with mechanical filters to avoid *Philometra* sp. and periodical freshwater baths for *Entobdella* sp. were enough to avoid parasite proliferation.

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