Implications of continuous feeding on oxygen demand in an agastric short intestine fish (Teleostei: *Atherinopsidae*)

Implicaciones de la alimentación continua sobre la demanda de oxígeno en un pez agástrico con intestino corto (Teleostei: *Atherinopsidae*)

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ABSTRACT. Continuous illumination and increased feeding periods have enhanced growth in commercial fish species. However, oxygen consumption under these conditions has yet to be previously assessed in agastric short-intestine fish. This study evaluated *Chirostoma estor* (Atherinopsidae) oxygen consumption under three specific feeding and light regimes: resting-unfed, routine-fed, and continuous light/feeding. Fish in the continuous light/feeding condition presented oxygen consumption levels 60 and 20% higher than resting-unfed and routine-fed fish, respectively. The latter implies that constant high oxygen supply levels are required to satisfy the demand for *C. estor* raised under these high growth rate conditions. The present study shows the relevance of determining oxygen consumption levels during continuous illumination and extended feeding periods to consider optimal oxygen levels required to achieve better performance indicators, such as the growth and welfare of atherinopsids and species with similar digestive configurations.
Keywords: *Chirostoma estor*, continuous illumination, frequent feeding.

RESUMEN. La iluminación continua y el aumento de frecuencias alimenticias han mejorado el crecimiento de peces en cultivo. Sin embargo, el consumo de oxígeno en estas condiciones no se ha evaluado en peces agástricos de intestino corto. Este estudio evaluó el consumo de oxígeno de *Chirostoma estor* bajo tres regímenes específicos de alimentación y luz: reposo sin alimentación, alimentación de rutina y alimentación/luz continua. En este último tratamiento los peces presentaron niveles de consumo de oxígeno de 60 y 20% más altos que los peces en reposo y con alimentación rutinaria, respectivamente. Esto implica que se requieren niveles elevados y constantes de suministro de oxígeno para satisfacer la demanda de *C. estor* en cultivo en condiciones crecimiento acelerado. El presente estudio muestra la relevancia de determinar los niveles de consumo de oxígeno bajo protocolos de crecimiento acelerado para lograr mejores indicadores de desempeño de otras especies con configuraciones digestivas similares.
Palabras clave: *Chirostoma estor*, iluminación continua, alimentación frecuente.

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INTRODUCTION

New world silversides (Atherinopsidae) such as the Mexican Pike silverside *Chirostoma estor* and the Argentinian Pejerrey *Odontesthes bonariensis*, have promising aquaculture potential due to their cultural and gastronomic relevance (high market value), low trophic status (higher sustainability), and nutraceutical value (high omega 3 content), all of which are ideal characteristics for future candidate aquaculture species (FAO 2010, Martínez-Chávez *et al.* 2018, Martínez-Palacios *et al.* 2020). However, a significant throwback for their commercial success has been the slow growth observed when feeding protocols developed for other farmed species are applied (Martínez-Palacios *et al.* 2006, Miranda *et al.* 2006, Ross *et al.* 2008, Somoza *et al.* 2008). To our knowledge no other fish species in the world has the frequent feeding requirements of *C. estor* (~1 hour). Therefore, species-specific husbandry protocols are required based on their biology (*i.e.*, feeding and digestive physiology), which is considerably different from those of currently farmed species (Ross *et al.* 2006, Toledo-Cuevas *et al.* 2011, Martínez-Palacios *et al.* 2019, Melo *et al.* 2023).

Nevertheless, growth performance in *C. estor* has been significantly (70%) improved experimentally by understanding its basic biology and applying biotechnological tools such as continuous illumination and frequent feeding (Martínez-Palacios *et al.* 2002, 2004, Martínez-Chávez *et al.* 2014, Corona-Herrera *et al.* 2022, Melo *et al.* 2023). Despite the higher metabolic activity under these enhanced-growth conditions, oxygen consumption and its operational implications (life support design) have yet to be considered to develop species-specific grow-out systems for Atherinopsids at different production scales (rural, semi-intensive, and intensive) in Mexico and South America, where these species are cultured with basic life support system technology. Therefore, this work aims to contribute knowledge that can be directly applied to bioengineering design for the aquaculture of Atherinopsid species.

MATERIALS AND METHODS

Despite no organisms where euthanized for sampling purposes, all experimental work was carried out in accordance with The European Parliament and The Council of the European Union, 2010/63/EU for animal experiments and the guidelines for the accommodation and care of animal used for experimental and other scientific purposes (The Commission of The European Communities 2007), adhering to the reduce, reuse and recycle principle.

Open respirometer set-up

Because *Chirostoma estor* are schooling fish, they are easily stressed when placed individually in conventional closed respirometer chambers, reflecting unnatural conditions which do not occur under culture. Thus, an experimental system was developed with open respirometers, as previously reported for other species (Brougher *et al.* 2005), providing a more practical approach to determine occurring oxygen consumption levels under culture. Three open respirometers (replicates) were used to place live fish in each trial; they consisted of a PVC tank of 2.60 m in length, 0.4 m wide, and 0.2 m high, with 168 L of capacity. Water originating from a deep well was supplied by two interconnected 300 L tanks, the first used as a constant-pressure header tank and the second to achieve maximum water oxygen saturation using multiple air stones. A compartment with a perforated wall allowed a constant laminar flow rate (1.5 L min^{-1}, set with a needle valve) to ensure adequate water mixing across the water column in each respirometer. A 1/16 hp pump (Boyu® model GX4P-6500T Guangdong, China) was used to transfer water to a cooler (Resun® model CW0500 Longgang, Shenzhen, China) for controlling the temperature at 24 ± 1°C (Martínez-Palacios *et al.* 2002).

The dissolved oxygen (DO) measurements were obtained *in situ* with an oxygen meter (YSI®, model 550A Yellow Springs, Ohio, USA), calibrated following manufacturing standards (at saturation, considering the local altitude, 1898 MAMSL). Measurements were taken by placing the electrode sensor at the influent and effluent in a counter-flow manner, thus stabilizing measurements for every respirometer. A fourth respirometer (without fish) was used as blank to consider the oxygen diffusion at the water surface, and the possible Biological Oxygen Demand (BOD), which values were subtracted from the oxygen consumption levels of each respirometer replicate.

Experimental design

Oxygen consumption of fish under three different feeding regimes was tested: a) resting-unfed, defined as the activity of fish under fasting and natural lighting and swimming conditions; b) routine-fed, defined as the activity of fish fed 6 times a day and under natural lighting conditions; and c) continuously fed, defined as the metabolic activity of fish fed every hour during 24 h under continuous illumination (24 h). Eight-month-old juvenile fish (*C. estor*) (41.4 ± 0.5 g) were obtained from stocks bred in captivity at Morelia, Michoacán, México, and allocated randomly in each respirometer. Formulated diets contained 42% protein, 5% lipid, and 4560 kcal kg^{-1}, the currently used for this species (Martínez-Palacios *et al.* 2007).

Feeding regimes

Resting-unfed oxygen consumption: A total biomass of 500 ± 36 g of fish was introduced in each of three open respirometers (replicates) and acclimated for two weeks. During this period, fish were fed *Artemia sp*. nauplii twice per day (9:00 and 14:00 h) *ad libitum* the first week, a common practice for this species, which promotes feeding and an adequate transition to experimental conditions. The following week fish were fed to apparent satiation 6 times per day, on the hour, from 9:00 to 14:00 h with the formulated diet. After the acclimation, DO measurements were taken every hour for 24 hours on fasting fish. Fish were exposed to a natural photoperiod (approximately 13 h light: 11 h dark).

Routine-fed oxygen consumption: At the end of the previous trial, fish remained in the respirometers for one week for another acclimation period and were also fed with *Artemia sp*. nauplii *ad libitum* twice a day. The following week, fish were started on a day feeding regime of 6 times per day (9:00 to 14:00 h on the hour) to apparent satiation with the formulated diet. DO levels in this trial were measured hourly before feeding over a 24 h period starting on the third week. As before, fish were exposed to a natural photoperiod (approximately 13 h light: 11 h dark).

Continuous illumination and feeding oxygen consumption: Following the routine-fed trial, fish were acclimated under continuous illumination (24 h light: 0 h dark, with four 40 watts fluorescent bulbs in each treatment) and fed 24 h a day on the hour with the same formulated diet during 5 days. On day six, under the same conditions, DO levels were measured over a 24 h period hourly before feeding. Photoperiod was maintained under continuous illumination during this trial.

Oxygen consumption rates were calculated for each trial using the following equation (Forsberg 1997): $M = (DO_{in} - DO_{out}) \times Q \times B^{-1}$

Where M is the weight-specific oxygen consumption rate (mg O_2 kg^{-1} h^{-1}), DO $_{in}$ and DO $_{out}$ are the dissolved oxygen concentrations (mg L^{-1}), in the inlet and outlet water, respectively, Q is the water flow (L min^{-1}), and B is fish biomass (kg) in the tank. The mean oxygen consumption rate (mg O_2 kg^{-1} h^{-1}) and daily total oxygen consumption (mg O_2 kg^{-1} day^{-1}) were determined.

Data were analyzed for normality and homoscedasticity using Shapiro-Wilks and Bartlett's tests. Repeated measures ANOVA analyses were done for feeding regimes to determine significant differences in oxygen consumption with a *post hoc* Tukey analysis (α = 0.05).

RESULTS AND DISCUSSION

Extended photoperiods (18L:6D) or continuous illumination are common tools to enhance growth in commercial fish species (Taranger *et al.* 2010, Hansen *et al.* 2017). Under these illumination conditions, prolonged feeding periods and locomotion activities can significantly increase metabolic rates. Published information about oxygen consumption under these conditions is limited to gastric species such as Turbot and Nile Tilapia (Imsland *et al.* 1995). On the other hand, stomachless-short intestine fish are interesting models with high aquaculture potential being low trophic consumers and frequent feeders due to their feeding habits and digestive configuration (Day *et al.* 2011, Vital-Rodríguez *et al.* 2017, Martínez-Chávez *et al.* 2018). In this work, the oxygen consumption of *C. estor* juveniles was evaluated under different light and feeding conditions in open respirometers, providing a first insight into oxygen demand levels for commercial grow-out applications in a gastric short-intestine fish. Total daily oxygen consumption rates in this study presented significant differences within the tested conditions. The mean daily oxygen consumption and total daily oxygen consumption rates in routine-fed and continuous light/feeding treatments were 34% and 60% higher than in resting-unfed conditions, respectively. When comparing feeding treatments, continuous light/feeding increased the mean daily oxygen consumption and total daily oxygen consumption rates by around 20% compared to routine-fed conditions (Table 1).

Table 1. Oxygen consumption of *Chirostoma estor* for resting-unfed, routine-fed, and continuous light/feeding conditions.

Treatment	Mean daily oxygen consumption rate (mg O_2 kg^{-1} h^{-1})	Total daily oxygen consumption (mg O_2 kg^{-1} day^{-1})
Resting-unfed	116.5 ± 5.62^{b}	2826.0 ± 86.82^{c}
Routine-fed	156.3 ± 4.99^{a}	3776.0 ± 82.62^{b}
Continuous light/feeding	186.1 ± 6.99^{a}	4467.0 ± 167.70^{a}
P value	0.0072	0.0017

Different letters indicate significant differences between treatments (P ≤ 0.05).

For practical comparative purposes, oxygen consumption data from continuous light and frequent feeding treatment were plotted together with resting-unfed and routine-fed treatments (Figure 1). Fish under continuous light/feeding had constantly higher mean oxygen consumption levels (oscillating between 160-200 mg O_2 kg^{-1} h^{-1}) as compared to those in the resting and routine trials (1.4-fold and 1.6-fold respectively), except during the acrophase, which is directly associated to feeding (13-16 h) where oxygen levels reached those of continuous feeding (Figure 1). Feeding every hour under continuous illumination increases oxygen consumption in juvenile *C. estor* by 20% compared to routine-fed treatment. Increased oxygen consumption under continuous illumination compared to a controlled photoperiod was also found in turbot (Imsland *et al.* 1995). However, in contrast to the current study, a diel profile remained, possibly because the feeding regime in turbot was restricted to the natural photophase. Because feeding frequency is necessary to achieve high growth rates in this agastric short-intestine fish (Corona-Herrera *et al.* 2022, Melo *et al.* 2023), the higher oxygen demand under continuous light and feeding should be considered for the life support systems design of this and other atherinopsids under culture.

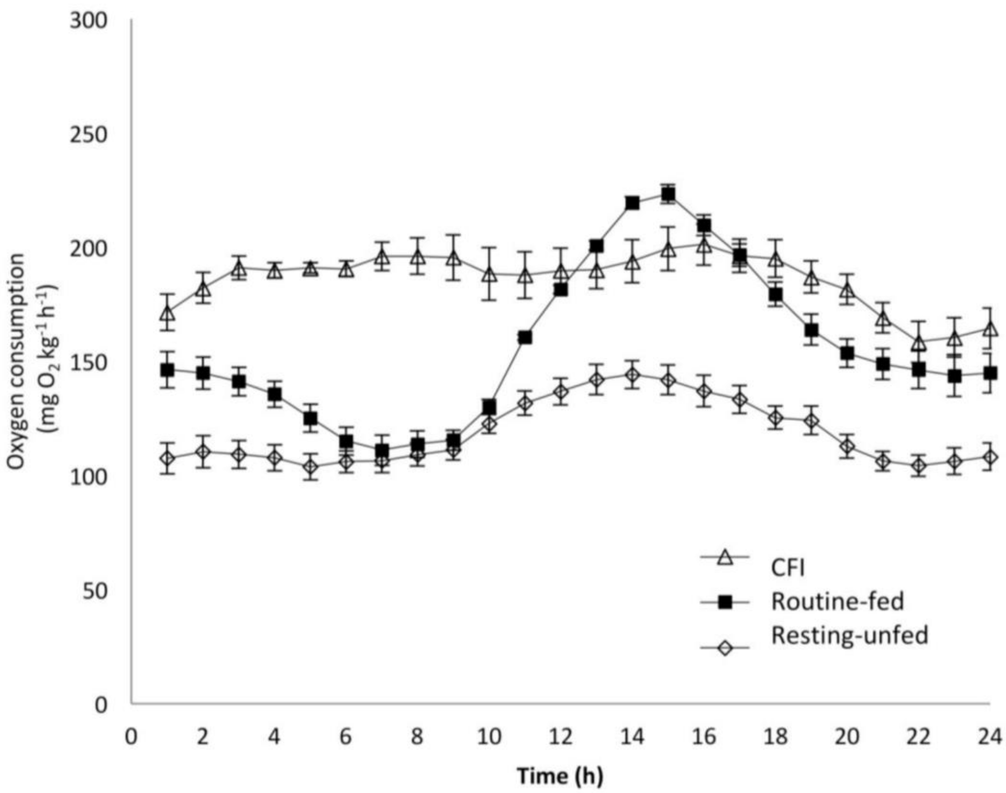

Figure 1. Oxygen consumption profiles of juvenile *Chirostoma estor* under resting-unfed, routine-fed and continuous light/feeding conditions at 24 ± 1°C (mean ± S.D). Feeding time points for routine-fed and continuous feeding and illumination are not shown.

The fact that fish in this study were observed to accept feed every hour for 24 hours stands out the frequent feeding capacity of *C. estor* compared to other digestive fish models (gastric or agastric with long intestine), which may feed less frequently. Thus, a frequent feeding strategy is crucial in these digestive models to achieve adequate growth performances (Corona-Herrera *et al.*

2022, Melo *et al.* 2023) and explains the apparent slow growth previously reported (Martínez-Palacios *et al.* 2006, Miranda *et al.* 2006, Ross *et al.* 2008, Somoza *et al.* 2008).

Interestingly, *C. estor* under resting-unfed treatment showed a similar diel oxygen consumption profile to the routine-fed condition of lower amplitude (Figure 1). This suggests the presence of an anticipatory feeding rhythm activity, established by the previous acclimation-feeding period as reported in other teleosts (Madrid *et al.* 2001, López-Olmeda and Sánchez-Vázquez 2010), this, however, would need further confirmation.

Dissolved oxygen levels need to be particularly monitored in the culture of *C. estor* under fast growth conditions (continuous illumination/feeding) as they consume around 20% more oxygen than fish fed 6 times a day under a natural photoperiod. The latter has logistic and technical implications, such as programable automatic feeders, lighting technology, and continuous oxygen monitoring to achieve maximum growth in *C. estor* culture under appropriate welfare parameters.

The results provide valuable information about *C. estor* oxygen requirements under two feeding and photoperiod regimes. This information is valuable for novel agastric short-intestine species cultured under continuous illumination and extended feeding regimes. This basic bioengineering information can be used for designing intensive rearing facilities for the culture of *C. estor* and other atherinopsids.

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CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

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