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**Semblanzas Ictiológicas**  
**Leandro Andrés Miranda**



**Hugo L. López**  
**y**  
**Justina Ponte Gómez**

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# Semblanzas Ictiológicas

Leandro Andrés Miranda



Leandro Miranda en la granja piscícola *YasudaFarmFish*, Japón, 2001

**Hugo L. López y Justina Ponte Gómez**

**ProBiota**  
División Zoología Vertebrados  
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**Imagen de tapa**

Muestreo en la laguna de Chascomús, 2010

*El tiempo acaso no exista. Es posible que no pase y sólo pasemos nosotros.*

**Tulio Carella**

*Cinco minutos bastan para soñar toda una vida, así de relativo es el tiempo.*

**Mario Benedetti**

## **Semblanzas Ictiológicas**

A través de esta serie intentaremos conocer diferentes facetas personales de los integrantes de nuestra “comunidad”.

El cuestionario, además de su principal objetivo, con sus respuestas quizás nos ayude a encontrar entre nosotros puntos en común que vayan más allá de nuestros temas de trabajo y sea un aporte a futuros estudios históricos.

Esperamos que esta iniciativa pueda ser otro nexo entre los ictiólogos de la región, ya que consideramos que el resultado general trascendería nuestras fronteras.

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## Cuestionario

- Un libro:** El Origen de las Especies
- Una película:** The wall
- Un CD:** Mentiras Piadosas
- Un artista:** Norberto Napolitano (Pappo)
- Un deporte:** futbol y buceo deportivo
- Un color:** azul y oro
- Una comida:** asado
- Un animal:** orca
- Una palabra:** tolerancia
- Un número:** 31
- Una imagen:** El David
- Un lugar:** playa Pardelas, Península Valdés
- Una estación del año:** verano
- Un nombre:** Guadalupe
- Un hombre:** René Favaloro
- Una mujer:** Evita
- Un personaje de ficción:** Meteoro
- Un superhéroe:** Ultraman



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Aquaculture

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## Development of sperm cryopreservation techniques in pejerrey *Odontesthes bonariensis*

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### ABSTRACT

The pejerrey, *Odontesthes bonariensis*, is being considered for aquaculture due to its high demand and market price. Reproduction and larviculture studies have demonstrated the feasibility of mass breeding production, and techniques that prolong life and increase gamete viability can aid in the culture development of this species. In this regard, the main objective of this study was to develop freezing protocols for pejerrey sperm. For this purpose, two extenders (Ex1, a modified Mowab solution (127 mM NaHCO<sub>3</sub>, 159 mM sucrose, 0.025 g/ml reduced glutathione; pH: 8; osmolality 400 mOsm/kg) and Ex2, a saline based solution (250 mM NaHCO<sub>3</sub>, 100 mM trehalose; pH: 8; osmolality 450 mOsm/kg) were developed. Dimethyl sulfoxide (DMSO) and ethylene glycol (EG) were added at 10% as cryoprotectants and two types of containers were used: cryotubes (1 ml of volume) and French straws (0.250 ml). Cryopreservation was made without equilibration time using dry ice or liquid nitrogen. The results obtained by freezing pejerrey semen with dry ice showed that all the combinations tested were viable because high motility indexes (among 4 or 5) and good fertility percentages (between 40% and 50%) were obtained after thawing. However, the fertilization percentage obtained with control semen was significantly higher (80%). No significant statistical differences were observed on the fertilization percentages between experimental combinations or the containers used. In the case of liquid nitrogen as freezing method, it was possible to obtain for all the combinations motility indexes among 3 or 4 after thawing. In the case of fertilization trials, similar percentages (around 80%) were found for control semen and for the majority of cryopreserved samples. However, statistically lower results (among 40%–60%) were found for Ex1 DMSO (cryotubes) samples and for Ex1 EG samples in both containers. In summary, the feasibility to cryopreserve pejerrey sperm was demonstrated for the first time using simple and practical protocols.

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### 1. Introduction

The pejerrey *Odontesthes bonariensis* is a euryhaline fish that naturally inhabits the continental waters of the Patagonian Region of Argentina (Lópezot et al., 2001). Over the last years, decreasing stocks of regional populations associated with overfishing and pollution (Berasain et al., 2005) targeted the pejerrey as a potential species for aquaculture (Miranda and Somazzi, 2001; Somazzi et al., 2000). Furthermore, the pejerrey is very appreciated by the local fisheries and citizens, and for this reason, it has been introduced in many water bodies across Argentina and other countries, such as Japan where it's being cultured at a commercial scale (Mitsui, 2001).

On this regard, pejerrey intensive culture methods had been successfully achieved in Argentina (Miranda et al., 2005; Somazzi et al., 2005, 2006). Despite these advances, many issues still need to be

solved in order to establish pejerrey farms in the region (Somazzi et al., 2006). For instance, pejerrey breeders present a marked asynchrony between spawning intervals, with a relatively low fecundity ranging from 300 to 400 (Strümann, 1995; Miranda et al., 2005). In the case of captive pejerrey males, the volume of expressible sperm is very low (100–200  $\mu$ l) from 3 to 4-year-old (Strümann et al., 1994) and the sperm concentration ranged between 3.5 and 6.5  $\times 10^6$  cells/ml (Miranda et al., 2005).

Overall, the current scenario, dictates that a large number of broodstock fish is necessary to be maintained, since efficient hatchery operation requires uniformity in larvae size, quantity and quality (Somazzi et al., 2006).

Recently in order to improve the current breeding procedures, some experiments were performed using environmental and hormonal treatments to increase pejerrey sperm volume and synchronize spawning females (Miranda et al., 2001, 2005, 2006). Although this path is pointing in good direction there's a different approach that could result more advantageous and, indeed, complementary with the pharmacological treatments.

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## General and Comparative Endocrinology

Journal homepage: [www.elsevier.com/locate/ygcen](http://www.elsevier.com/locate/ygcen)Effects of global warming on fish reproductive endocrine axis, with special emphasis in pejerrey *Odontesthes bonariensis*Leandro Andrés Miranda<sup>A,\*</sup>, Tomás Chalde<sup>A</sup>, Mariano Eliso<sup>A</sup>, Carlos Augusto Strüssmann<sup>B</sup><sup>A</sup> Laboratorio de Endocrinología y Acuicultura, Instituto de Investigaciones Biotecnológicas (IIB) – Tecnología de Alimentos (FOAG) (CONICET-UNBA), Av. Irigoyen 1801, San Carlos de Bariloche (8300) Neuquén, Buenos Aires, Argentina<sup>B</sup> Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, 4-1-7 Kanari, Minato, Tokyo 108-8501, Japan

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## ABSTRACT

The ongoing of global warming trend has led to an increase in temperature of several water bodies. Reproduction in fish, compared with other physiological processes, only occurs in a bounded temperature range; therefore, small changes in water temperature could significantly affect this process. This review provides evidence that fish reproduction may be directly affected by further global warming and that abnormal high water temperature impairs the expression of important genes throughout the brain–pituitary–gonad axis. In all fishes studied, gonads seem to be the organ more readily damaged by heat treatments through the inhibition of the gene expression and subsequent synthesis of different gonadal steroidogenic enzymes. In view of the feedback role of sex steroids upon the synthesis and release of GnRH and GTH in fish, it is possible that the inhibition observed at brain and pituitary levels in treated fish is a consequence of the sharp decrease in plasma steroid levels. Results *in vitro* studies on the inhibition of pejerrey gonad aromatase expression by high temperature corroborate that ovary functions are directly disrupted by high temperature independently of the brain–pituitary axis.

For the reproductive responses obtained in laboratory fish studies, it is plausible to predict changes in the timing and magnitude of reproductive activity or even the total failure of spawning season may occur in warm years, reducing annual reproductive output and affecting future populations.

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## 1. Introduction

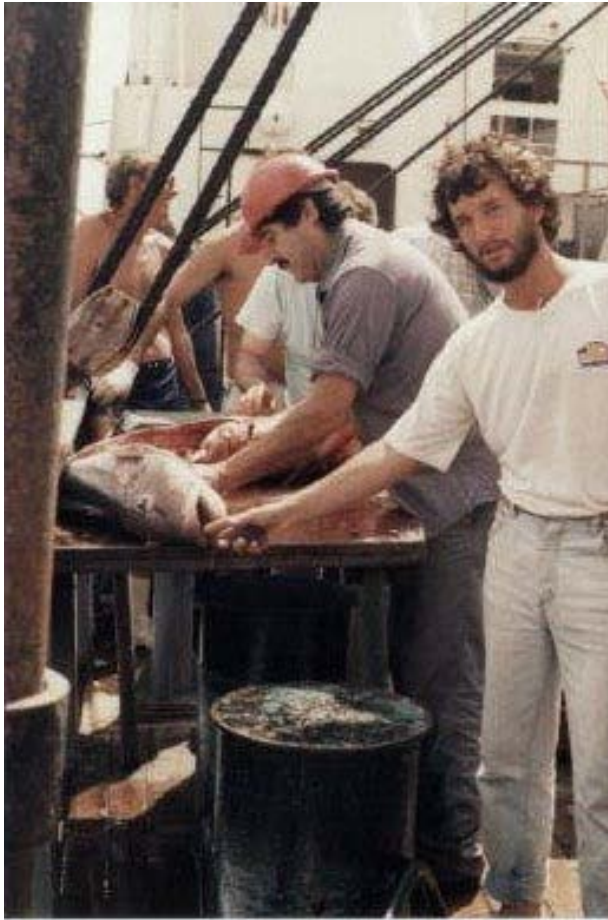
Temperature is one of the most important physicochemical variables that determine the overall functioning of aquatic communities (Ficko et al., 2007; Jørgensen et al., 2010; Mostj et al., 2009; Pörtner and Farrell, 2008). All physiological processes within an organism occur within a limited temperature range, which may differ depending on the molecular and cellular mechanisms associated with each particular process (Pörtner and Farrell, 2008). In this sense, species are adapted to a certain range of temperature variation, depending largely on the geographic area where they have evolved.

Fish, as well as other ectothermic organisms, have a body temperature “virtually” equal to that of its environment, so any variation in this variable will affect them directly (Ficko et al., 2007). Thus, the temperature in aquatic ecosystems is a key variable in the geographical distribution of different fish species (Cunha et al., 2008; Perry et al., 2005), and any temporary change in its normal patterns could generate consequences such as changes in

abundance, spatial distribution and even extinction (Ficko et al., 2007).

The reproduction in fish, compared with other physiological processes, only occurs in a bounded temperature range (Pörtner and Farrell, 2008) therefore, small changes in water temperature could significantly affect this process (Van der Kraak and Parkhurst, 1997; Zilba et al., 2010; Zucchetta et al., 2012). In temperate climates, the thermal conditions that allow reproduction in most fish usually occur seasonally and, together with day length (Meganathan et al., 2010) determine the reproductive seasonality of different fish species (Fig. 1). This, in an evolutionary form is interpreted as a mechanism associated with the selection of environmental conditions that increase the likelihood of survival and development of the offspring (Bromage et al., 2001; Pörtner and Farrell, 2008). In this context, abnormal water temperature conditions could generate a mismatch between reproduction and optimal environmental conditions for progeny development (Durant et al., 2007). Besides, shortening or even complete loss of the breeding season could also happen (Eliso et al., 2012a). It is important to note that any of these possible scenarios could generate a loss in the reproductive output of a given population, change its structure and may jeopardize its sustainability (Durant et al., 2007; Strüssmann et al., 2010).

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Campaña científica INIDEP en el Oca Balda, 1989  
Leandro Miranda, primero a la derecha

Pesca científica, INIDEP, 1989  
Leandro Miranda (derecha) y Fernando de la Torre (izquierda)





Muestreo de pejerreyes, Bioterio IIB-INTECH, 2011  
Leandro Miranda y Tomás Chalde



Muestreo en la laguna Salada de Pedro Luro, provincia de Buenos Aires, 2012  
Miembros del grupo Peces, Proyecto Pampa, CONICET; Leandro Miranda de pie, tercero desde la derecha





Instalación de boya meteorológica en la laguna de Chascomús, 2012  
De izquierda a derecha, Alejandro Vitale, Leandro Miranda y Dario Colautti

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