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An infection of *Gyrodactylus anguillae* Ergens, 1960 (Monogenea) associated with the mortality of glass eels (*Anguilla anguilla* L.) on the north-western Mediterranean Sea board of Spain

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ABSTRACT

The association of *Gyrodactylus anguillae* Ergens, 1960 with the glass eel stage of *Anguilla anguilla* (L.) (total body length 61.4 ± 4.9 mm; range 55–70) is reported from the north-western Mediterranean coast of Spain for the first time. A sample of 12,600 glass eels, caught by professional fishermen operating in the mouth of the rivers Fluvià, La Muga and Ter (north-east Spain), was subject to mortalities of ~1.75% of stock/day following transfer to a research facility. Subsequent losses over a 31-day period amounted to 56% of the initial stocked biomass. Although the moderate burdens of *G. anguillae*/host (20.2 ± 6; range 11–32) were the primary reason for a subsequent treatment, a simultaneous infection with *Trichodina jadranica* Raabe, 1958, *Trichodina anguillae* Wu, 1961 and *Ichthyophthirius multifiliis* Fouquet, 1876, makes it impossible to attribute the high mortality of glass eels in this case to a single pathogen. A histopathological examination of the gills of moribund fish showed them to be swollen, hyperplastic and necrotic. This study also redescribes *G. anguillae*, providing for the first time a full 27 character morphometric description of the attachment hooks, and importantly, a photographic record of the armature of the haptor and the male copulatory organ.

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1. Introduction

Wild stocks of the European eel, *Anguilla anguilla* (L.), are no longer considered sustainable, with levels of glass eel returns currently between 1 and 9% of those reported in the 1970s (ICES, 2010). The European eel is thus listed as a critically endangered species on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species and has been added to the Annex B of the Convention on International Trade in Endangered Species (CITES) (see Freyhof and Kottelat, 2008). Declining numbers have been attributed to climate change, habitat loss, overfishing, deterioration in water quality and disease (Feunteun, 2002). The reported loss of a significant number of glass eels in north-eastern Spain, possibly attributable to a *Gyrodactylus* infection, was therefore worthy of investigation.

The current study describes the mortality of glass eels, collected from the Fluvià, La Muga and Ter rivers, Catalonia, Spain, following their transfer to an aquarium facility and attempts to assess the possible role of the monogenean parasite *Gyrodactylus anguillae* Ergens, 1960 and other more opportunistic pathogens in the observed losses.

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2. Materials and methods

2.1. Collection and maintenance of hosts

During February 2009, a total of 12,600 glass eels (weight range on capture 110–300 mg, mean total body length 61.4 ± 4.9 (1 S.D.), range 55–70 mm) was caught in the mouths of the Fluvià, La Muga and Ter rivers (north-eastern Spain) using traditional, fixed, non-selective fish traps known in the Catalan language as "busso" (Lopez and Gisbert, 2009). Glass eels from this fishery, operated by professional fishermen, were collected at night over a period of three weeks using artisanal and rudimentary sorting methods, during which little care was taken with regard to glass eel welfare. During handling, the eels undergo high levels of stress, exacerbated by air exposure, hypoxia and skin lesions (Gisbert and Lopez, 2008). The eels were held in two 2 m^3 flow-through freshwater tanks in BASE VIVA SL, a fish hatchery located in Sant Pere Pescador (Girona, Spain), for a period of one month. During this time, eels were fed daily with non-enriched *Artemia* nauplii. Thereafter, animals were transported by road (4 h), in two 500 L fibreglass tanks (14 °C and 90% oxygen saturation) to the IRTA-Sant Carles de la Rapita (IRTA-SCR) facilities where they were acclimatised and grown on for river restocking purposes. Upon arrival, the fish were randomly distributed between two 2 m^3 circular tanks forming part of a recirculation system (IRTAMAR™)with constant aeration (dissolved oxygen 8–9 ppm, 90% saturation) and daily water renewal (40 L h^{-1}). During the two week acclimation period, the water temperature was incremented by 1 °C/day up to 19 °C. Water temperature, conductivity, pH and dissolved oxygen for the entire rearing period were 19.0 ± 1.0 °C, 2100 ± 200 μS cm^{-1}, 8.4 ± 0.3 pH and 8.5 ± 0.5 ppm O_2, respectively. Photoperiod was 12L:12D, with a light intensity of 80.1 ± 10.5 lux at the water surface. During the acclimation and rearing period, the glass eels were fed non-enriched *Artemia* nauplii (INVE EG, Belgium) combined with cod roe (*Gadus morhua* L.) to apparent satiation (10% body weight day^{-1} given in two rations).

2.2. Eel mortalities

No data on glass eel mortalities during their initial maintenance at the BASE VIVA SL facilities is available. The glass eels began dying shortly after their transfer to the research facility at the IRTA-SCR. In the first 8 days, daily losses of stock were approximately 1.75%. Macroscopic examination of a random sub-sample of approximately 20 eels revealed the presence of monogeneans on the skin (precise parasitic burden undetermined). In an attempt to control the worms, 100 ppm static formaldehyde treatments were conducted in each rearing tank at days 9, 11 and 14 post-arrival to the new rearing facilities. In addition, a 1 mg L^{-1} mebendazole (Sigma) 24 h treatment (Buchmann et al., 1987) was given to control worms on day 15. Despite these chemical interventions, the treatment regime did not reduce the rate of eel mortality. Water chemistry parameters were not affected by chemical treatments. Thirty one days after the transfer of the eels to the new rearing facilities, a total of 7050 glass eels was lost, representing 56% of the starting stock.

2.3. Morphometric identification of the monogeneans

On day 16 post-transfer and 24 h following the mebendazole treatment, a random sub-sample of 12 glass eels was fixed in 10% neutral buffered formalin and sent to the Parasitology Research laboratory at the Institute of Aquaculture, University of Stirling, UK for evaluation. Each eel was examined macroscopically, with any monogeneans adhering to the external surfaces being counted and carefully removed using mounted triangular surgical needles (size 16, Barber of Sheffield, UK). The heads of five eels were removed and processed for histology following standard procedures for wax embedding and haematoxylin and eosin staining of 5 μm sections. The skin, gills, nares and the mouth cavity of the remaining eels were examined for ectoparasites under an Olympus SZ30 stereomicroscope. Fifty monogeneans were rinsed in distilled water and then prepared as whole mounts using ammonium picrate glycerine (APG) according to the method of Malmberg (1970). The tissue enclosing the attachment hooks of a further 30 distilled water rinsed specimens was removed using the proteolytic digestion method detailed in Paladini et al. (2009). This method works well on fresh or ethanol fixed material, but the digestion process takes considerably longer on formalin fixed parasites. As only formalin-fixed material was available for the current study, the 10 best APG whole mounts were selected for morphometric analysis. In total, 27 point-to-point morphometric measurements were made on the attachment hooks of each specimen from images captured using KS300 (ver. 3.0) (Carl Zeiss Vision GmbH, 1997) image analysis software, a JVC KY-F30B 3CCD video camera mounted on an Olympus BH2 microscope with a 2.5× interfacing lens and a 100× oil immersion objective lens. In addition, the total dimensions of each specimen, the haptor, the pharynx and the male copulatory organ (MCO), where present, were recorded. Images of the MCO and proteolytic digested hooks were captured with a Zeiss AxioCam MRc digital camera interfacing with an Olympus BH2 compound microscope using a 0.75× lens and MRGrab 1.0.0.4 (Carl Zeiss Vision GmbH, 2001) software. The morphometric measurements made on the attachment hooks of each specimen follow those described in Shinn et al. (2004), but also include the length and width of the dorsal bar. The tabulated measurements are expressed as the mean ±1 standard deviation, followed by the range in parentheses.

3. Results

3.1. Macroscopic and histopathological examination

Each glass eel (n = 12) was found to be parasitised by a single monogenean species, *G. anguillae* (prevalence = 100%), on the skin and gills and within the nares and the mouth (20.2 ± 6 (11–32) parasites/host). This number of recovered parasites may represent an underestimate, as the fish studied had already been subjected to formalin and mebendazole treatments at the time of sampling.

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