

# First record of grey rockcod *Lepidonotothen squamifrons* larvae in the Amundsen Sea, Antarctic: implication for a new spawning ground and early life characteristics

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## Research Article

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# Abstract

Grey rockcod, *Lepidonotothen squamifrons* is widespread throughout the Antarctic, however, its early life information is little known. Highly abundant larvae of grey rockcod were collected during a zooplankton survey conducted in the Amundsen Sea polynyas in March of 2022. This is the first record of grey rockcod larvae found at high latitudes of the Southern Ocean. The otolith microstructure analysis provided more insight into the early life characteristics of grey rockcod. A total of 41 individuals with a standard length of 9.6–15.3 mm were estimated of 6–11 days. Multiple primordia were first recorded in the otolith. Gompertz model was well fitted to the age–length data, indicating a mean daily growth rate of  $0.66 \text{ mm day}^{-1}$ . Larval hatching occurred at a size of 4.08 mm and was concentrated on a short period, at early March. Compared with the population at lower latitude, grey rockcod in the Amundsen Sea polynyas showed later hatch time, smaller hatch size, and faster growth rate. Therefore, we speculate that Amundsen Sea polynyas are potential spawning grounds for grey rockcod. The revealed spatial differences in early life characteristics could contribute to understanding the life history strategy and adaptation of grey rockcod.

## Introduction

The Amundsen Sea, one of the most productive and dynamic pelagic systems in the Southern Ocean (Arrigo 2003, Alderkamp et al. 2015), is located between the Ross Sea and the Bellingshausen Sea in West Antarctica and contains abundant sea ice as well as coastal polynyas (Fragoso and Smith 2012). Polynyas is a seasonal open water circulation area surrounded by sea ice and is an important component of the Antarctic marine system (Massom and Stammerjohn 2010). Once the polar night ends, solar radiation is able to penetrate the water in time to produce the earliest warming and irradiation that can boost earlier seasonal phytoplankton production (Jiang et al. 2016). Therefore, polynyas provide an ideal, almost completely free of ice habitats for polar marine life, and the Amundsen Sea polynya is one of the most productive polynyas. A recent study reported a spawning ground for *Pleuragramma antarctica* in the Amundsen Sea polynyas, indicating that the area is suitable for fish breeding and larval growth (Duan et al. 2022b). Sea ice also affects the biodiversity and distribution of species, the environmental conditions of polynyas will dramatically change with the rapid melting of glaciers. The polynya has turned into a climate-sensitive area in the Antarctic Ocean since sea ice extent in the Amundsen Sea has been decreasing over the last few decades (Arrigo and Alderkamp 2012, Yager et al. 2012). The above characteristics make Amundsen Sea polynyas a window to study the influence of local and global climate changes on fish species, especially fish larvae, and further studies on fish community and life history in this area are required.

Grey rockcod *Lepidonotothen squamifrons*, which synonymised names *Notothenia squamifrons* (Miya et al. 2016), is an endemic species in the Southern Ocean, mainly occurring at 250–350m on the continental shelf. The species has a circumpolar distribution along the Antarctic continental shelf, especially in the South Georgia Islands and the Ross Sea also lives in the waters around the sub-Antarctic islands such as the Kerguelen Islands (Duhamel et al. 2014). Similar to Channichthyidae fishes, grey rockcod has a long

pelagic larval and juvenile phase and the life span connects the pelagic and benthic layers. Larvae and juveniles of grey rockcod and related (Kock, 1992) *Nototheniidae* have been the most common species in the continental shelf ichthyoplankton community (Granata et al. 2002).

Grey rockcod was once exploited as one of the economic fish species and the population of grey rockcod in the Atlantic and Indian Oceans is the main target of commercial fishing, but the degree of exploitation of the resource differs between the two regions. Grey rockcod was commercially exploited on the Kerguelen shelf from 1970 to 1990 (CCAMLR 2004). The catches composed 22.3% of the total reported landings from 1970 to 1994, and reached peak catch in 1972 (Kock 1992, Ainley and Blight 2009). With the catches rapidly declined since 1982 (Duhamel et al. 2011), the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) prohibited direct commercial fishing for grey rockcod in 1989 (Duhamel 1981, CCAMLR 1989, Kock 1992). However, the current knowledge on the life history of grey rockcod is quite limited, especially in early life characteristics such as larval duration and incubation time. These early life characteristics strongly influence recruitment success and the connectivity between populations. To our best knowledge, only La Mesa et al. (2017) studied the early life strategies of grey rockcod in the southern Scotia Arc and further demonstrated inter-specific interactions among three *Lepidonotothen* fishes. The lack of relevant knowledge could limit better understanding of population dynamics and stock assessment.

In this study, using larvae samples of grey rockcod collected in the Amundsen Sea polynya for a zooplankton survey, we aim to estimate the size, timing of hatching, and growth rate of the larval stage through otolith microstructure analysis. The result could fill a gap in the distribution record of Grey rockcod larvae within the Antarctic Circle and add important information about early life history of this species.

## Materials And Methods

### Field sampling

The cruise of the 37th Chinese Antarctic Research Expedition was aboard the polar research vessel *Xue Long 2*. The pelagic fish survey was conducted in the Amundsen Sea and Amundsen Sea polynya in March 2020. A 0.565m<sup>2</sup>-opening bongo net fitted with 505- $\mu$ m mesh was used to survey pelagic zooplankton (0-200m). The trawl speeds were approximately 2.5 knots and the towing time was about 30 minutes. A Flow-Meter (Hydro-Bios) was equipped with bongo net to record the amount of filter water. Only two stations sampling used bongo net were conducted in the polynyas area of the Amundsen Sea, and both of these resulted in the presence of larvae.

### Sample processing

The fish larvae individuals were sorted and preserved in 95% alcohol, and morphological taxonomic identification was performed in the Fisheries Resources Laboratory after the return of the research vessel. Identification was based on the shape and position of the eyes, fins, and pigmentation (Koubbi et al.

1990)(Fig. 2). The standard length of each larva was measured to the nearest 0.1 mm. Body shrinkage due to preservation was considered to be negligible. Then, sagittal otolith was dissected under a stereomicroscope (JSZ5A) with fine needle, and embedded in epoxy drops (Buehler, EpoThin 2) on glass slides with the medial side down. To enhance the readability of increment patterns, otoliths were hand-ground with 1200 grit and 2500 grit water-resistant sandpaper until the daily growth increments could be observed clearly under a light microscope, and then polished with 0.05  $\mu\text{m}$  alumina powder. The otoliths images were obtained under 1000 $\times$ oil immersion magnification using a microscope system (OLYMPUS BX53 and DP74).

## Ageing

Otolith increments were counted from the primordium to the edge. As La Mesa et al. (2017) assumed, the otolith increments were regarded laid down daily. Thus, daily age for each individual was the number of all increments observed along the counting path with the assumption that the first increment was to be laid down at hatching. Each individual was aged twice with ImageJ software and otoliths in which the counts differed were read again.

## Data analysis

The larval hatch date distribution was back-calculated by subtracting age estimates from the date of capture.

Gompertz model was fitted based on the age-length data to describe larval growth as La Mesa et al. (2017) suggested. The selected model is summarized in the following equation:

$$SL = L_{\infty} \exp(- \exp(- G(t - t_0))),$$

where  $L_{\infty}$  is the asymptotic length,  $G$  is the instantaneous growth rate at the inflexion point of the curve  $t_0$  (i.e. the age at which absolute growth rate begins to decrease), and  $t$  is the age of fish. The absolute daily growth rate ( $g$ ,  $\text{mm day}^{-1}$ ) at age  $t$  was calculated applying the following equation:  $g_t = GL_t (\ln L_{\infty} - \ln L_t)$ , where  $G$  is the instantaneous growth rate,  $L_{\infty}$  and  $L_t$  are the fish length at the asymptote and age  $t$ , respectively. Larval size at hatching (i.e.  $SL$  at age  $t=0$ ) was also calculated.

The statistical analysis and graph plotting were all performed using R (version 4.0.3).

## Results

A total of 41 grey rockcod larvae were collected in two stations, and the abundance demonstrated a high level compared with the known spawning grounds around Kerguelen Islands (Koubbi et al. 2000) (Table 1, Fig. 1). The developmental stages of the larvae ranged from preflexion of the notochord ( $N=15$ ) to postflexion of the notochord ( $N=26$ ).

Table 1 **Sample information of grey rockcod conducted in the Amundsen Sea in 2020-2021.**

Station	Latitude	Longitude	Standard length mm	Age (days)	<i>N</i>	Abundance (inds/1000m <sup>3</sup> )	Date
A10-5	70.987°S	84.992°W	10.0-15.3	7-10	15	11	14 <sup>th</sup> Mar. 2021
A10-6	70.519°S	84.980°W	9.6-14.7	6-11	26	20	

The sagittal otoliths of larvae showed a discoid shape. Within the core area, the primordium was an optically dense and irregular discoidal area, encircled by a distinct check (hatch ring) (Fig 3a). Few individuals contain multiple primordia and hatch checks irregularly change (Fig 3b). Off the hatch ring, the increment pattern appears as alternating dark and light zones.

Overall, all larvae were successfully aged through increment counts, age estimates ranged from 6 to 11 days. Then, the hatch date was back-calculated, grey rockcod hatched around early March with a pronounced peak in 5<sup>th</sup> Mar. (Fig. 4)

Gompertz growth curves fitted to age-length data was determined as follows (Fig 5):

$$SL = 20.13 \exp(-\exp(-0.13(t - 3.6))),$$

The absolute daily growth rate equation of grey rockcod larvae is:

$$g_t = 0.13L_t (\ln 20.131 - \ln L_t),$$

Based on this function, the larval size at hatching was estimated to be approximately 4.08 mm. The mean absolute daily growth rate calculated was 0.66mm day<sup>-1</sup>, and the maximum length of larvae is 20.13mm.

## Discussion

This study investigated the early-life history traits of grey rockcod in the Amundsen Sea polynyas. Although the sampling effort is relatively small, the survey discovered newly hatched larvae of grey rockcod with high level of abundance (Koubbi et al. 2000). It is the first record that grey rockcod larvae have been found at high latitudes. Compared with previous study in the southern Scotia Arc (Duhamel and Ozouf-costaz 1985, Damerau et al. 2012, Gregory et al. 2013), grey rockcod larvae of this study showed high abundance with different hatching date. The hatch date was also almost half a month later than that in the southern Scotia Arc. In addition, the previous survey discovered distribution record for grey rockcod adults around this area (Matallanas and Olaso 2006). Therefore, considering the influence

of the Antarctic Circumpolar Current, we speculate that Amundsen Sea polynyas are potential spawning grounds for grey rockcod where a separate population inhabits. However, because of the survey time, the specimens did not cover all recruitment cohorts (only summer) in this study (Koubbi et al. 2000). Thus, a further survey is needed to fully understand the spawning pattern of grey rockcod in the Amundsen Sea polynyas.

The otolith microstructure analysis and then fitted growth function provide more insight into early life characteristics of grey rockcod. Multiple primordia were first recorded in the otolith of grey rockcod, which is featured by Channichthyidae fishes (Duan et al. 2022a) and not reported in other *Lepidonotothen* fishes. The phenomenon needs further study to confirm whether environmental condition leads to such abnormality. On the other side, the reconstructed early life history actually revealed latitude differences. As a trade-off, fecundity decreases, and egg size at spawning, and larvae at hatching increase with latitude in the Antarctic at intra- and inter-specific levels. This spawning strategy could maximize the individual fitness and survival potential of offspring in the early stages. In addition, fecundity difference was reported between populations of grey rockcod, which could be a result of food availability and temperature. In the Amundsen Sea polynya, the estimated larval size at hatching was smaller than the estimated size of 5.8mm in the southern Scotia Arc and the observed size of 6.0mm in South Shetlands (La Mesa et al. 2017, La Mesa et al. 2020). In contrast, the instantaneous growth rate at the inflexion point and absolute daily growth rate was higher than the values ( $0.053 \text{ day}^{-1}$  and 0.05–0.60 mm/day) in the southern Scotia Arc. According to the latitude trend, grey rockcod in the Amundsen Sea polynya should demonstrate low fecundity and larger hatch size. We speculate that the high primary productivity in the Amundsen Sea polynya may account for the observed discrepancy. Better food availability promoted growth rate and then increase survival potential for larvae.

In conclusion, the present study provides novel distribution information for grey rockcod in early life stages. The result further highlights the importance of the Amundsen Sea polynya in fish spawning and nursery. The revealed spatial differences in early life characteristics could contribute to understanding the life history strategy and adaption of grey rockcod, providing a specimen to specifically study the influence of environmental conditions on the early growth of fish.

## **Declarations**

### **Acknowledgement**

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### **Author contributions**

HG conducted the experiments, analyzed the data and wrote the manuscript. CZ conducted the experiments, and edited the manuscript. MD assisted sample processing. JL, WZ, collected the fish samples. YT designed and reviewed the manuscript. All authors read and approved the final manuscript.

**Conflict of interest.** The authors declare that they have no conflict of interests and that all applicable institutional, national, or international guidelines for the use and care of animals were strictly followed in the present study.

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## Figures

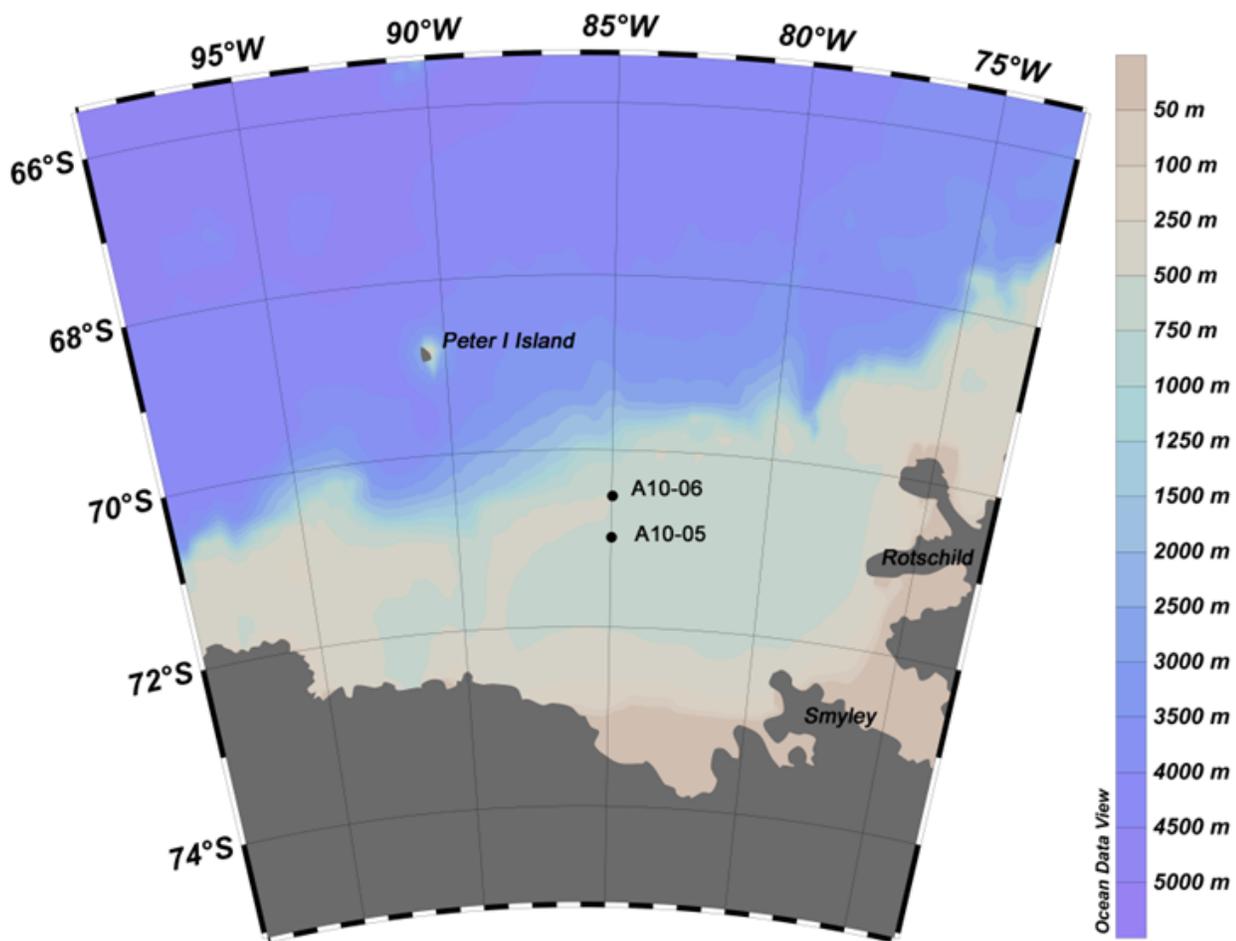


Figure 1

Map of sampling sites in the Amundsen Sea



Figure 2

Specimen of grey rockcod larva collected in the Amundsen Sea

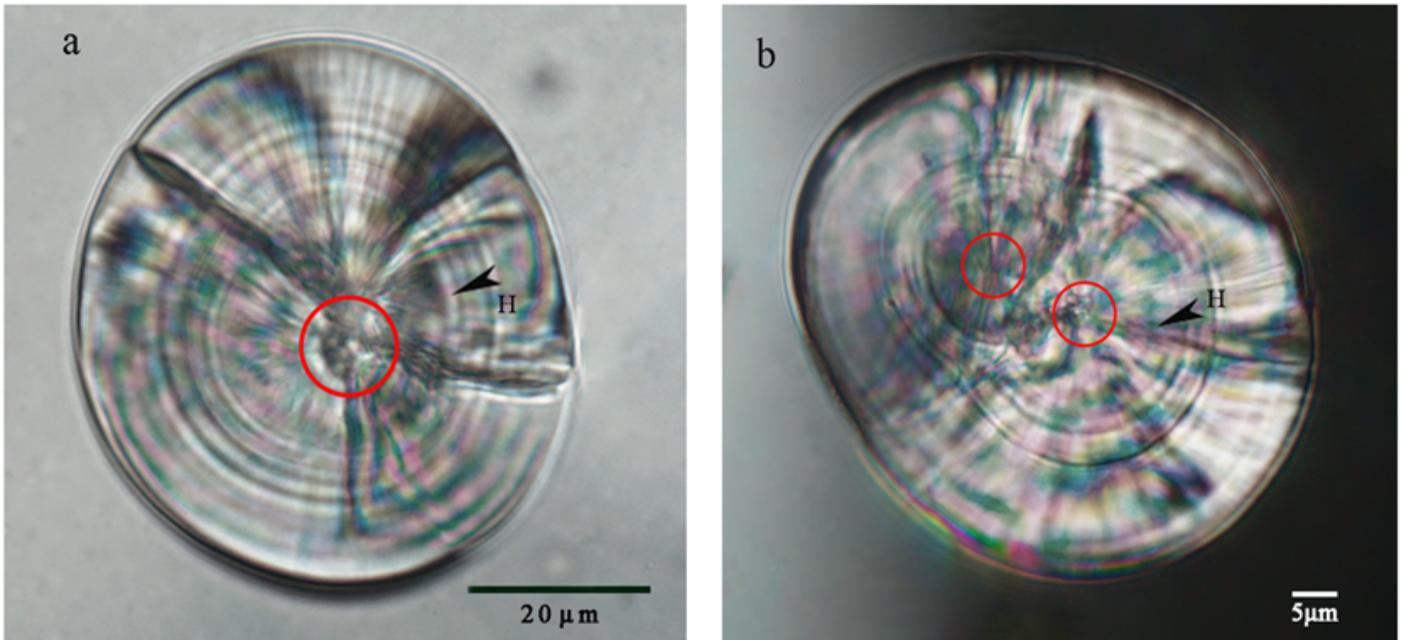


Figure 3

Photographs of otoliths of grey rockcod larvae. Hatch check and primordiums on the otolith are indicated by black arrows (H) and red circles respectively.

a. Otolith containing single primordium, SL=13.4mm

b. Otolith containing multiple primordia, SL=12.4mm

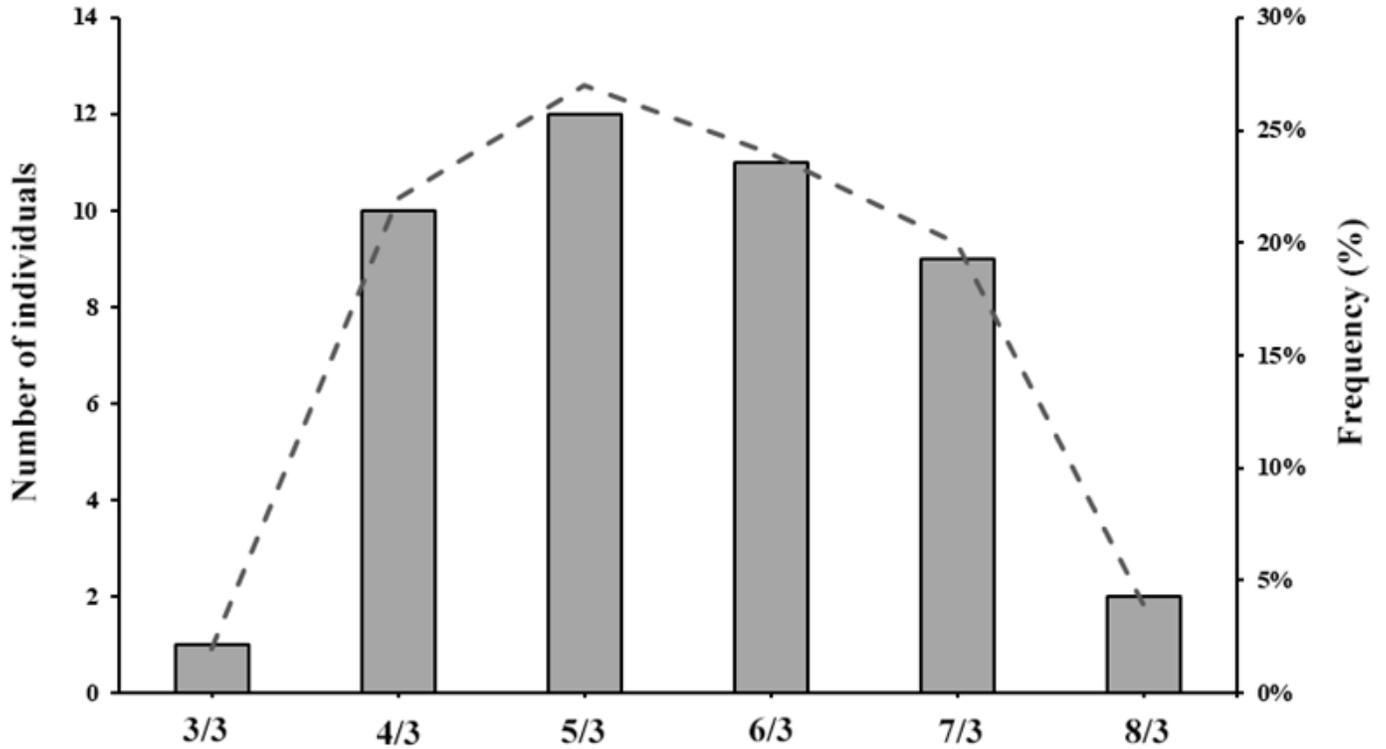


Figure 4

Hatching date distribution for early larvae grey rockcod back-calculated from age estimates and date of capture.

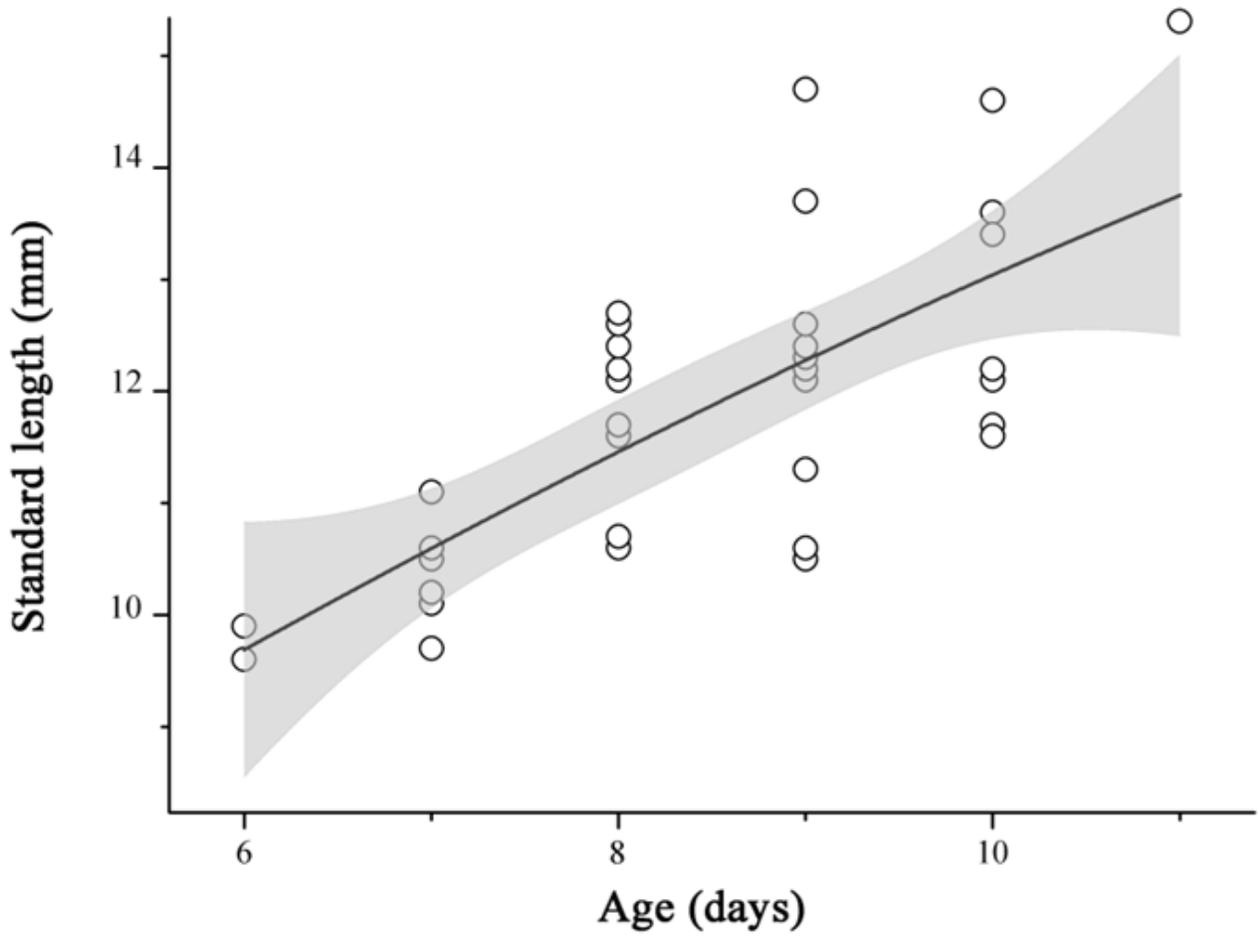


Figure 5

The estimated Gompertz growth curve of the grey rockcod larvae. The shaded gray area represents the 95% confidence interval.