

The use of otolith length and weight measurements in age estimations of three Gobiidae species (*Deltentosteus quadrimaculatus*, *Gobius niger*, and *Lesueurigobius friesii*)

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Abstract: Age estimations were made using otolith length and weight measurements from 3 goby species in the central Aegean Sea: the four-spotted goby (*Deltentosteus quadrimaculatus* Valenciennes, 1837), the black goby (*Gobius niger* Linnaeus, 1758), and Fries's goby (*Lesueurigobius friesii* Malm, 1874). A total of 266 individual four-spotted gobies, 283 black gobies, and 124 Fries's gobies provided otolith samples for analysis. The relationships between fish age and otolith length and fish age and otolith weight were determined, as were the relationships between total length and otolith length and total length and otolith weight. Results indicated that the relationship between fish age and otolith weight was the most suitable for estimating the age of the studied species. The relationships were estimated at $t = 0.338W - 0.199$ for the four-spotted goby, $t = 0.147W - 0.085$ for the black goby, and $t = 0.400W - 0.619$ for Fries's goby. The present paper also provides drawings of the species' otoliths to aid in the identification of the species. This research provides information that can be applied not only for age estimation but also for prey identification and estimation of the size of prey ingested by piscivorous predators.

Key words: Alternative age estimation method, four-spotted goby, black goby, Fries's goby

Üç Gobiidae türünün otolit boyu ve ağırlığının yaş tahmininde kullanımı (*Deltentosteus quadrimaculatus*, *Gobius niger* ve *Lesueurigobius friesii*)

Özet: Üç kayabalığı türünün; dört benekli kayabalığı (*Deltentosteus quadrimaculatus* Valenciennes, 1837), kömürcü kayabalığı (*Gobius niger* Linnaeus, 1758) ve kayabalığı (*Lesueurigobius friesii* Malm, 1874), orta Ege Denizi'ndeki otolit boyu ve ağırlığının yaş tahmininde kullanımı araştırılmıştır. Toplamda; 266 adet dört benekli kayabalığı, 283 adet kömürcü kayabalığı ve 124 adet kayabalığı otoliti analiz edilmiştir. Balık yaşı - otolit boyu ve balık yaşı - otolit ağırlığı arasındaki matematiksel ilişki tespit edilmiş, ayrıca buna ek olarak total boy - otolit boyu ve total boy - otolit ağırlığı arasındaki ilişkiler de belirlenmiştir. Balık yaşı - otolith ağırlığı arasındaki ilişkilerin çalışılan türlerin yaş tahmini için en uygun eşitlik olduğu görülmüştür. Dört benekli kayabalığı, kömürcü kayabalığı ve kayabalığı için bu eşitlikler sırası ile $t = 0,338W - 0,199$, $t = 0,147W - 0,085$ ve $t = 0,400W - 0,619$ olarak tespit edilmiştir. Ayrıca bu çalışma, otolitten tür tayinine yardımcı olması için türlere ait otolit çizimlerini de içermektedir. Bu çalışmada sunulan bulgular sadece yaş tahmininde değil, ayrıca balıkla beslenen canlıların avladığı türlerin ve avın boyunun tahmini için de kullanılabilir.

Anahtar sözcükler: Alternatif yaş tahmin metodu, dört benekli kayabalığı, kömürcü kayabalığı, kayabalığı

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Introduction

The estimation of fish age and growth is fundamental to fisheries' biology and management (Summerfelt and Hall, 1987; Morales-Nin, 1992; Campana, 2001). Age determination in fish can be carried out using the anatomical method (counting the regular growth marks formed in hard tissues such as scales, otoliths, vertebrae, spines, and tail bones), length-frequency analysis (monitoring the progression through time of the identifiable modes in size classes), or direct measurement (the measurements of growth rate of specific specimens extrapolated to the stock as a whole. The marking and subsequent recapture of fish or the monitoring of the growth of captive fish of known ages are 2 examples of direct methods) (Morales-Nin, 1992).

The anatomical method is the most preferred age-determination method for bony fishes, and the otolith is often the most preferred hard structure used for this. Otoliths are primarily composed of calcium carbonate, and the depositions of annual growth rings formed in this tissue are caused by seasonal changes in the environment. All bony fishes (Osteichthyes) have 3 pairs of otoliths called the sagitta, asteriscus, and lapillus. The sagitta is the largest otolith in most bony fishes, although, in the otophysan fishes (Cypriniformes, Siluriformes, Characiformes, and Gymnotiformes) the asteriscus are the largest (Berra and Aday, 2004; Froese and Pauly, 2009). Because of its size and distinct growth rings, the sagittal otolith is often preferred for age determination.

Although the anatomical method process is reliable most of the time, it may give rise to erroneous age determinations in older fish and in otoliths with excessive calcium carbonate accumulation (Metin and Kınacıgil, 2001). An accurate age determination depends on the reader's skill, which is closely associated with experience and an amount of bias in the age estimations of different readers (Sandeman, 1969; Ernst et al., 1995). In addition, the amount of manpower and time spent in determining age with this method is considerable (Cardinale et al., 2000). For these reasons, alternative methods of determining the ages of fish are being sought, with particular emphasis on potential methods that would require less effort and deliver a higher level of precision. One of these alternative methods makes

use of the relationship between the size and weight of the otoliths and the age of the fish (Metin and İlkyaz, 2008). Härkönen (1986) found that there is a high correlation between fish length and otolith length and that this relationship is usually linear. Furthermore, Brander (1974) claimed that otolith weight has a direct relationship to the age of the fish. Several researchers have found a strong relationship between fish length and otolith length (Hoşsucu et al., 1999; Morat et al., 2008) and between fish age and otolith length/weight (Lou et al., 2007; Zorica et al., 2007; Metin and İlkyaz, 2008). These relationships have also been used in research on the stomach contents of piscivorous fishes (Morley and Belchier, 2002), marine birds (Johnson et al., 2006), and marine mammals (Pierce and Boyle, 1991) for prey identification as well as size and age determination.

The family Gobiidae is the largest family and contains the smallest size of vertebrates of the marine fishes. The family is mostly distributed in tropical and subtropical areas, such as shallow coastal marine waters and around coral reefs. Many goby species are popular as aquarium fish. Although the maximum length of the family is 50 cm, most are below 10 cm. There are 1578 goby species existing in the world and the species can be found inhabiting fresh, brackish, and marine waters. There are 74 goby species in the Mediterranean Sea and Black Sea, and 33 species are found in Turkish waters (Miller, 1986; Miller, 1990; Nelson, 2006; Froese and Pauly, 2009).

The objective of this study was to use otolith length and weight to determine age in specimens from 3 Gobiidae species: the four-spotted goby (*Deltentosteus quadrimaculatus* Valenciennes, 1837), the black goby (*Gobius niger* Linnaeus, 1758), and Fries's goby (*Lesueurigobius friesii* Malm, 1874). These data may also be used by researchers studying the food habits of piscivorous predators in order to determine the size of fishes. In addition, we have provided drawings of the otoliths to aid in the identification of the species.

Materials and methods

The samples (*Deltentosteus quadrimaculatus*, *Gobius niger*, and *Lesueurigobius friesii*) used in the study were collected from trawl hauls conducted

in İzmir Bay (central Aegean Sea, 38°40'N, 26°31'E and 38°2'N, 26°8'E) by the R/V *Egesüf* (26.8 m in length, 463-HP engine, and 110 t in gross weight); the sampling period was from July 2004 through June 2007. The trawl surveys were performed using a conventional bottom trawl on sandy and muddy bottoms in water 30 to 70 m deep. The cod-end was knotless, diamond-shaped mesh and made of polyamide (PA) material with a stretched mesh size of 22 mm.

The total length (TL) of each fish was measured and recorded with 1-mm precision. The sagittal otoliths of 266 four-spotted gobies, 283 black gobies, and 124 Fries's gobies were removed. The otolith lengths (L) were measured with an electronic caliper with a precision of 0.01 mm, while the weights (W) were measured with electronic scales accurate to 0.0001 g (0.1 mg). The otoliths were removed in pairs in case of any possible damage or loss during the measuring process. The sagittal otolith pairs were cleaned and stored in dry conditions inside the microplate, and measurements were always obtained from undamaged otoliths.

Age determination was performed using a stereoscopic zoom microscope under reflected light against a black background. To increase the viewing clarity, otoliths were placed inside a drop of glycerin. Opaque and transparent rings were counted, and 1 opaque zone together with 1 transparent zone was considered to be 1 annual increment. Some otoliths were cut and polished for age estimation. They were imbedded in polyester molds and cut with an IsoMet low-speed saw. Both sides were then polished with sandpaper (types 400, 800, and 1200) before finally being polished with 3, 1, and $\frac{1}{4}$ μ particulate alumina (Metin and Kinacıgil, 2001). Age readings were performed by 2 independent readers.

The relationships between total length and otolith length and total length and otolith weight were established as $TL = bL + a$ and $TL = aW^b$, respectively, where TL is total length (cm), L is otolith length (mm), W is otolith weight (mg), and a and b are the intercept and slope of the relationships. The relationship between fish age and otolith length was investigated by fitting the von Bertalanffy (1938) growth function to size-at-age data using standard nonlinear optimization methods (Metin and İlkyaz, 2008). The function

$$L_t = L_\infty (1 - e^{-k(t-t_0)})$$

and its transformed form

$$t = t_0 - \frac{\ln\left(-\frac{L_t}{L_\infty} + 1\right)}{k}$$

were used, where t is fish age (years), L_t is the otolith length (mm) at time t , L_∞ is the asymptotic otolith length (mm), k is the growth coefficient (year^{-1}), and t_0 is the hypothetical time at which otolith length is equal to 0 (years). The relationship between fish age and otolith weight was established as $t = bW + a$, where t is the age of the fish (years), W is the otolith weight (mg), and a and b are coefficients.

Results and discussion

In this study, a total of 266 individual four-spotted gobies were analyzed and a general view of the otolith is shown in Figure 1. It was determined that the four-spotted goby specimens ranged between 4.3 and 9.2 cm in total length ($\overline{TL} = 7.27$, $SE = 0.06$) (Table 1). The maximum total length previously reported by Miller (1986, 1990) for the eastern Atlantic was 8 cm, and our results therefore present a new maximum size for the species. The age compositions of the species were between I and V years old. The most dominant age class was III, comprising 50.0% of the specimens, followed by II (23.3%), IV (18.4%), and I and V (both approximately 4.1%). The average length of the otoliths was 3.11 ± 0.02 mm and the average weight was 9.50 ± 0.19 mg (mean \pm SE). A linear relationship between fish age and otolith weight was established as $t = 0.338W - 0.199$ ($R^2 = 0.992$). The relationship between fish age and otolith length was $t = -1.033 - [\ln(-L/4.087) + 1]/0.371$ ($R^2 = 0.994$). The coefficient of determinations of these equations were similar and both the relationship between fish age and otolith length and that of fish age to otolith weight can be used for age estimations for the species (Table 2 and Figure 2). There has been little previous data on the four-spotted goby and this study presents the first results reported for the species.

According to the results of otolith readings, the age distribution of the black goby was I-VII years of age and the length distribution ranged between 3.8 and 16.3 cm in total length ($\overline{TL} = 11.59$, $SE = 0.13$)

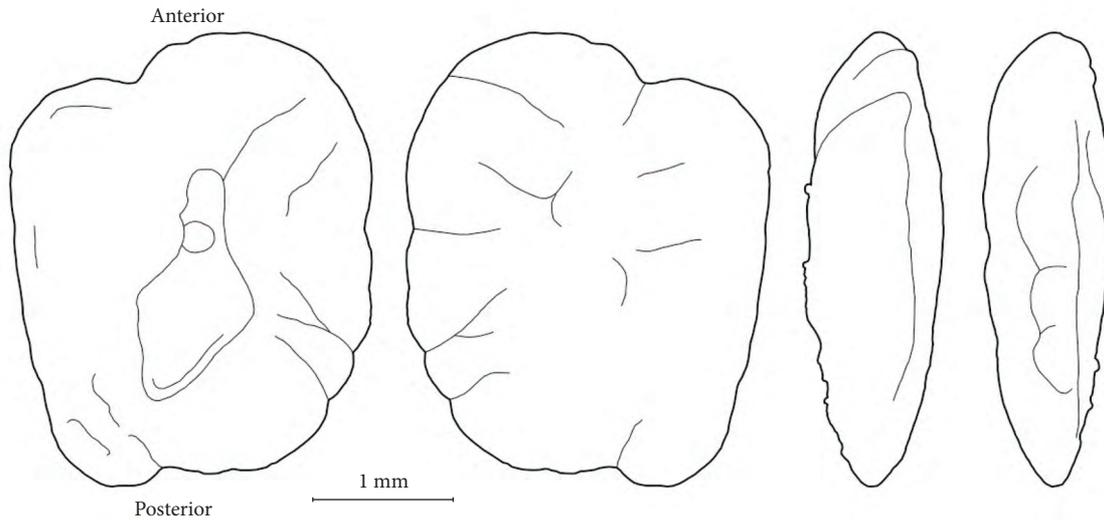


Figure 1. A general view of the four-spotted goby (*Deltentosteus quadrimaculatus*) otolith (TL = 8.2 cm, L = 3.44 mm, W = 10.8 mg, t = 4 years).

(Table 1). The age class III (43.1%) was dominant, followed by IV (22.6%), II (19.4%), V (7.1%), I (4.9%), VI (2.1%), and VII (0.7%). The maximum total length reported by Muus and Nielsen (1999) was 18 cm for the Scandinavian fishing area. Furthermore, Filiz and Toğulga (2009) reported a maximum size of 15.2 cm in total length from the same area as in this study. Although our results show that the species can reach VII years of age, previous studies have reported the maximum age as V for the Mediterranean (Fabi and Giannetti, 1985; Filiz and Toğulga 2009). This relatively large discrepancy was probably due to sampling differences (period and gear). The average length of the otoliths was 4.99 ± 0.05 mm and the average otolith weight was 22.99 ± 0.50 mg (mean \pm SE). This shows that the black goby had the largest and heaviest otolith of the studied species (Figure 3). The relationships between fish age and otolith length and fish age and otolith weight were $t = -0.131 - [\ln(-L/7.181) + 1]/0.393$ ($R^2 = 0.967$) and $t = 0.147W - 0.085$ ($R^2 = 0.986$), respectively (Table 2 and Figure 4). The coefficient of determinations of the age equations indicated that the relationship between fish age and otolith weight was the best for the age estimation of this species.

The total length of Fries's goby specimens ranged from 4.2 to 8.4 cm ($\overline{TL} = 6.02$, SE = 0.09) (Table 1).

Muus and Nielsen (1999) determined the maximum valid length as 13 cm for the North Sea and Baltic Sea; our samples were much smaller than that previously reported maximum length. In their own study of the species from the northern Aegean Sea of Turkey, Filiz and Bilge also reported the maximum length as 8.1 cm. Stergiou et al. (1997) reported that cases of shorter length (dwarfism) in benthic invertebrates inhabiting the eastern Mediterranean may also be valid for fish species. The difference in the maximum size of the species between the northern Atlantic and the eastern Mediterranean can probably be attributed to the variation in habitat, and the data presented in this paper should therefore only be used in the same geographic area. The ages of the Fries's goby samples ranged between I and V years, with 2-year-old fish being the most numerous (34.7%), followed by the I (31.5%), III (19.4%), IV (8.1%), and V (6.5%) classes. The average length of the otoliths was 2.74 ± 0.04 mm and the average weight was 7.14 ± 0.29 mg (mean \pm SE) (Figure 5). These results show that the species had the smallest and lightest otoliths of the 3 studied species. The fish age and otolith length relationship of the species was calculated as $t = -1.152 - [\ln(-L/3.628) + 1]/0.453$ ($R^2 = 0.890$), and the relationship between fish age and otolith weight was calculated as $t = 0.400W - 0.619$ ($R^2 = 0.981$) (Table

Table 1. Distributions of the four-spotted goby (*Deltentosteus quadrimaculatus*), black goby (*Gobius niger*), and Fries's goby (*Lesueurigobius friesii*) specimens in terms of age, item, fish length, otolith length, and weight.

	Age	n (%)	Fish length (cm)		Otolith length (mm)		Otolith weight (mg)	
			Min-max	$\bar{x} \pm SE$	Min-max	$\bar{x} \pm SE$	Min-max	$\bar{x} \pm SE$
<i>Deltentosteus quadrimaculatus</i>	I	11 (4.1)	4.3-5.5	4.40 ± 0.08	2.04-2.32	2.17 ± 0.03	2.7-4.2	3.38 ± 0.12
	II	62 (23.3)	4.9-7.1	6.27 ± 0.05	2.28-3.16	2.75 ± 0.03	3.9-10.4	6.47 ± 0.19
	III	133 (50.0)	6.7-8.1	7.47 ± 0.02	2.87-3.54	3.18 ± 0.01	6.6-13.5	9.70 ± 0.13
	IV	49 (18.4)	7.8-8.8	8.26 ± 0.03	3.28-3.72	3.49 ± 0.02	9.9-16.1	12.97 ± 0.20
	V	11 (4.1)	8.5-9.2	8.98 ± 0.05	3.44-3.84	3.65 ± 0.04	12.8-17.1	14.80 ± 0.37
	Σ	266 (100)	4.3-9.2	7.27 ± 0.06	2.04-3.84	3.11 ± 0.02	2.7-17.1	9.50 ± 0.19
<i>Gobius niger</i>	I	14 (4.9)	3.8-7.0	5.60 ± 0.32	1.72-3.64	2.57 ± 0.18	2.3-10.7	5.56 ± 0.74
	II	55 (19.4)	7.8-11.0	9.48 ± 0.18	3.04-4.93	4.20 ± 0.05	8.5-19.4	14.62 ± 0.34
	III	122 (43.1)	10.0-13.7	11.39 ± 0.11	4.33-6.04	5.00 ± 0.03	14.6-31.0	21.99 ± 0.29
	IV	64 (22.6)	12.0-14.7	13.14 ± 0.10	4.81-6.60	5.63 ± 0.05	21.3-39.4	29.24 ± 0.48
	V	20 (7.1)	14.0-14.9	14.46 ± 0.13	5.54-7.14	6.12 ± 0.09	30.4-43.5	36.99 ± 0.78
	VI	6 (2.1)	14.9-15.3	15.13 ± 0.09	5.82-7.08	6.31 ± 0.19	33.3-45.6	39.70 ± 1.72
	VII	2 (0.7)	15.4-16.3	15.85 ± 0.45	6.33-7.42	6.88 ± 0.54	45.6-47.3	46.45 ± 0.85
	Σ	283 (100)	3.8-16.3	11.59 ± 0.13	1.72-7.42	4.99 ± 0.05	2.3-47.3	22.99 ± 0.50
<i>Lesueurigobius friesii</i>	I	39 (31.5)	4.2-6.0	4.84 ± 0.05	2.04-2.66	2.26 ± 0.03	2.2-5.9	3.93 ± 0.14
	II	43 (34.7)	5.1-7.2	6.16 ± 0.04	2.30-2.97	2.67 ± 0.02	4.2-8.6	6.24 ± 0.15
	III	24 (19.4)	6.2-7.6	6.88 ± 0.04	2.97-3.39	3.17 ± 0.02	8.2-12.1	9.98 ± 0.21
	IV	10 (8.1)	6.6-7.7	7.24 ± 0.04	3.13-3.49	3.29 ± 0.04	10.3-13.3	11.52 ± 0.29
	V	8 (6.5)	7.0-8.4	7.73 ± 0.10	3.22-3.63	3.40 ± 0.05	11.6-17.7	13.55 ± 0.71
	Σ	124 (100)	4.2-8.4	6.02 ± 0.09	2.04-3.63	2.74 ± 0.04	2.2-17.7	7.14 ± 0.29

n: number of samples, %: rate in total, \bar{x} : average, SE: standard error, Σ : all samples.

Table 2. The relationship of total length - otolith length, total length - otolith weight, fish age - otolith length, and fish age - otolith weight for 3 goby species: the four-spotted goby (*Deltentosteus quadrimaculatus*), black goby (*Gobius niger*), and Fries's goby (*Lesueurigobius friesii*).

	Relationship	<i>a</i>	<i>b</i>	L_{∞}	<i>k</i>	t_0	SE_b	R^2
<i>Deltentosteus quadrimaculatus</i>	<i>TL-L</i>	-0.091	2.365				0.315	0.885
	<i>TL-W</i>	3.326	0.353				0.049	0.873
	<i>t-L</i>	1.268	0.690	4.087	0.371	-1.033	0.036	0.994
	<i>t-W</i>	-0.199	0.338				0.166	0.992
<i>Gobius niger</i>	<i>TL-L</i>	0.138	2.296				0.648	0.909
	<i>TL-W</i>	2.763	0.464				0.053	0.946
	<i>t-L</i>	2.331	0.675	7.181	0.393	-0.131	0.196	0.967
	<i>t-W</i>	-0.085	0.147				0.281	0.986
<i>Lesueurigobius friesii</i>	<i>TL-L</i>	0.035	2.186				0.275	0.922
	<i>TL-W</i>	3.127	0.344				0.049	0.914
	<i>t-L</i>	1.322	0.636	3.628	0.453	-1.152	0.130	0.890
	<i>t-W</i>	-0.619	0.400				0.254	0.981

L: otolith length (mm), *W*: otolith weight (mg), *t*: age (years), *TL*: total length of fish (cm), *a*: intercept of the relationship, *b*: slope of the relationship, R^2 : the coefficient of determination, SE_b : standard error of slope (*b*), L_{∞} : the asymptotic otolith length (mm), *k*: the growth coefficient (year⁻¹), t_0 : the hypothetical time at which otolith length is equal to 0 (years).

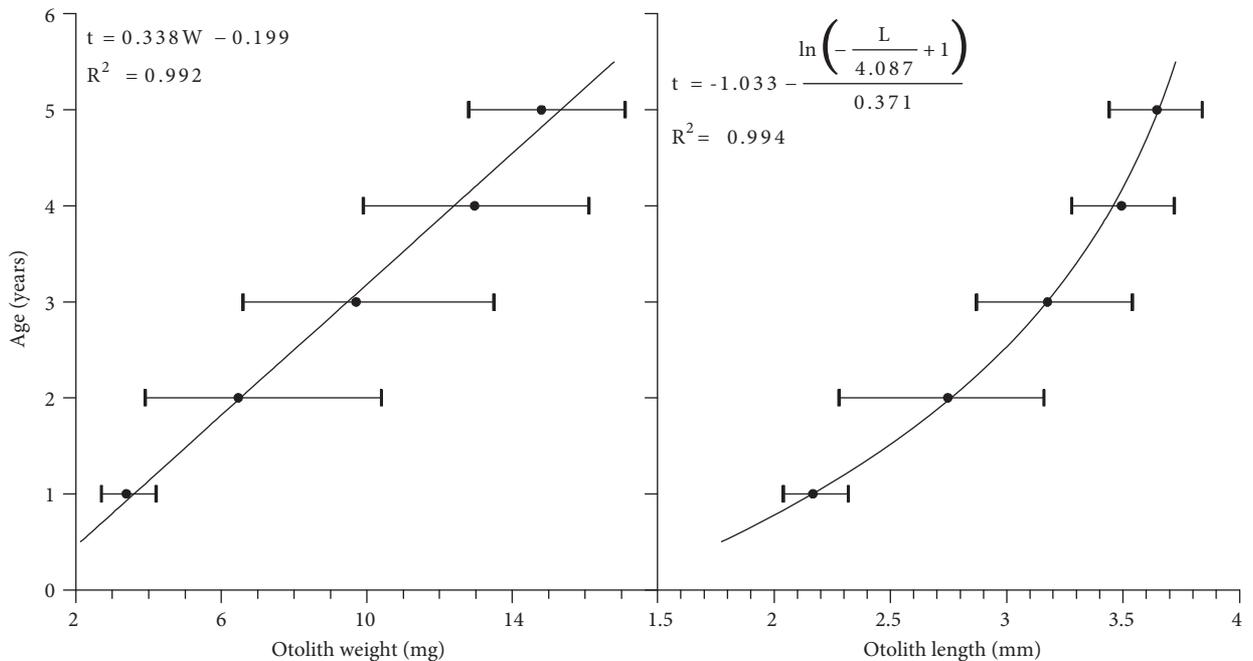


Figure 2. The relationship between fish age and otolith weight and fish age and otolith length with minimum and maximum size ranges for the four-spotted goby (*Deltentosteus quadrimaculatus*).

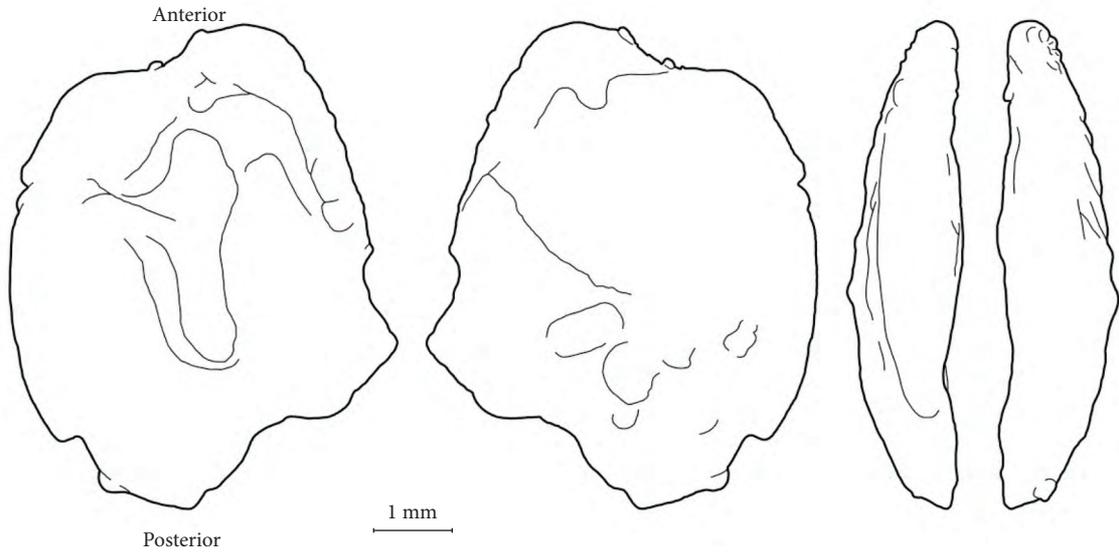


Figure 3. General view of the black goby (*Gobius niger*) otolith (TL = 16.2 cm, L = 6.34 mm, W = 44.2 mg, t = 6 years).

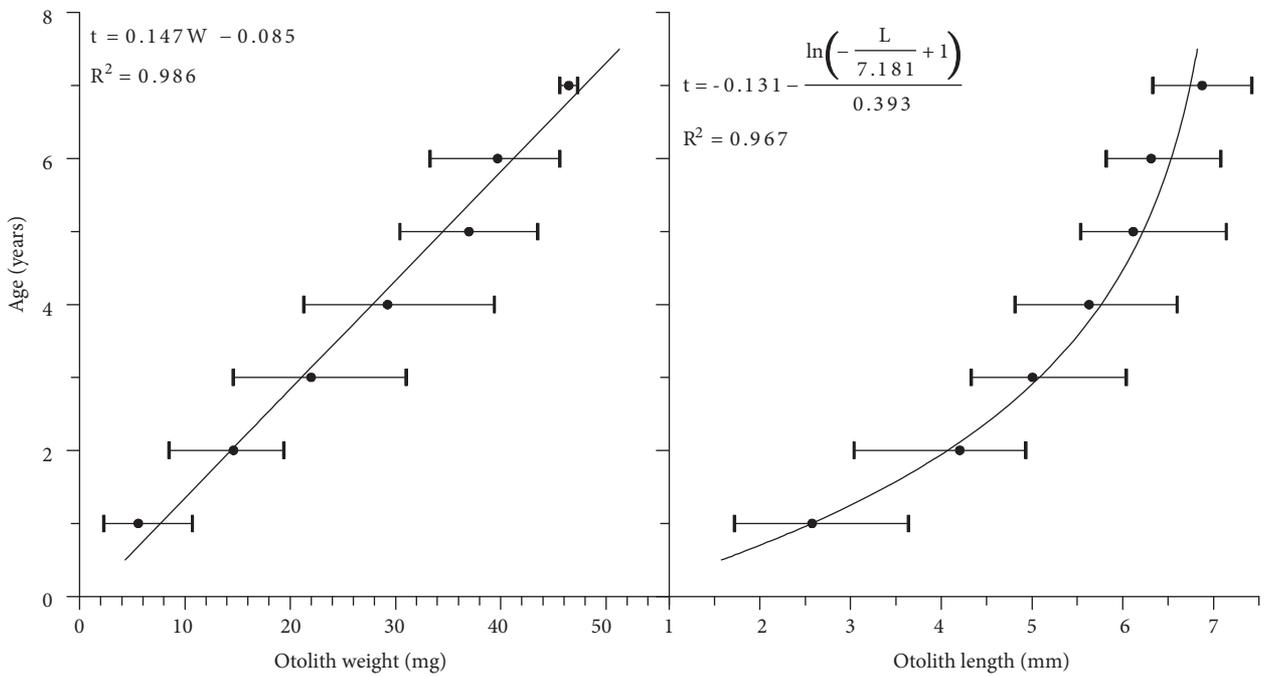


Figure 4. The relationship between fish age and otolith weight and fish age and otolith length with minimum and maximum size ranges for the black goby (*Gobius niger*).

2 and Figure 6). The coefficient of determinations of the age equations indicated that the equation of fish age in relation to otolith weight was the best for the age estimation of Fries's gobies.

In conclusion, the age of individual four-spotted gobies (*Deltentosteus quadrimaculatus* Valenciennes, 1837), black gobies (*Gobius niger* Linnaeus, 1758), and Fries's gobies (*Lesueurigobius friesii* Malm, 1874)

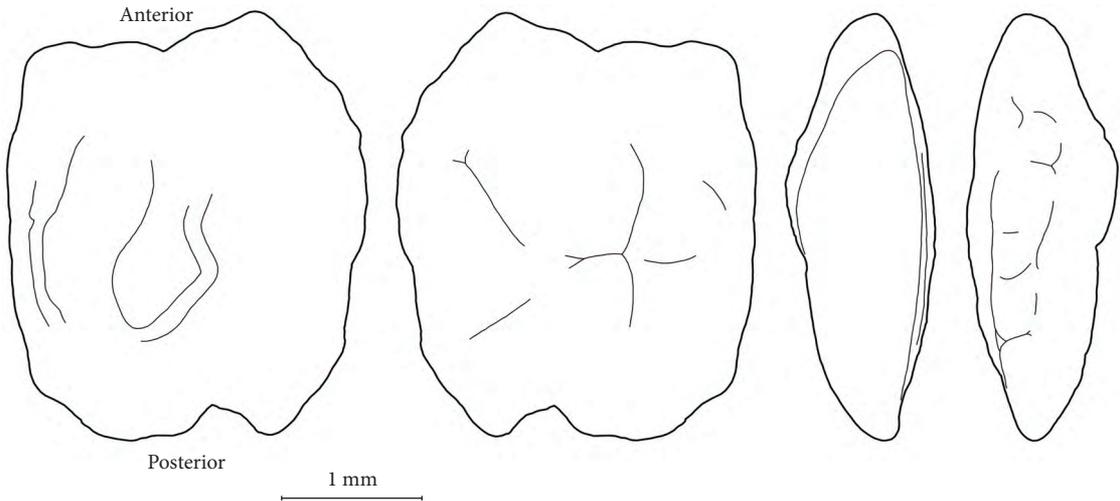


Figure 5. A general view of the Fries's goby (*Lesueurigobius friesii*) otolith (TL = 7.6 cm, L = 3.24 mm, W = 12.4 mg, t = 4 years).

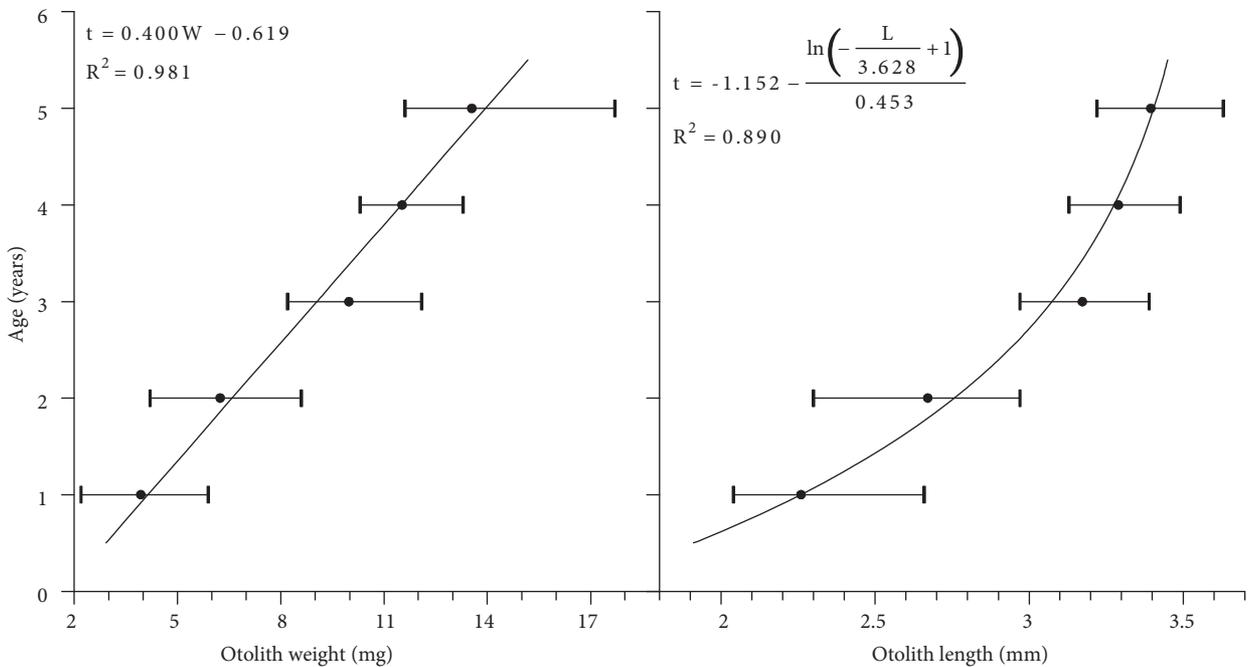


Figure 6. The relationship between fish age and otolith weight and fish age and otolith length with minimum and maximum size ranges for the Fries's goby (*Lesueurigobius friesii*).

can be estimated by using otolith length or weight. The relationship between fish age and otolith weight could be used in the age estimation of the 3 goby species with a high coefficient of determination and

practical usage. Additionally, this work provides information that can not only aid in age estimation but also provide some tools for the study of stomach contents (prey identification and sizing).

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