Italian Journal of Food Safety

https://www.pagepressjournals.org/index.php/ijfs/index

eISSN 2239-7132

Please cite this article as:

Tanjaoui Y, Dahani S, Bouchriti N, Oleya El Hariri O. **Occurrence of parasites in fishery species along the Moroccan coastline**. *Ital J Food Saf* doi:10.4081/ijfs.2025.13584

Submitted: 03-01-2025
Accepted: 26-02-2025

Occurrence of parasites in fishery species along the Moroccan coastline

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Key words: fishery species, Anisakidae, *Gymnorhynchus gigas*, xenomas, Morocco.

Contributions: YT, conducted the inspection of samples, analyzed the data, and drafted the manuscript. SD, NB, OEH, conceptualized the study, analyzed the data, supervised the study, and revised the manuscript.

Conflict of interest: the authors declare no competing interests.

Ethics approval and consent to participate: not applicable.

Availability of data and materials: the datasets used and analyzed during the current study are available upon reasonable request from the corresponding author.

Funding: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments: the authors thank all the team of the Food Safety Unit at Hassan II Agronomic and Veterinary Institute for their valuable support during all this work.

Abstract

The fishing industry in Morocco is a fundamental pillar of the national economy. However, it is important to acknowledge that fishery products can potentially transmit parasitic agents to humans. These parasites primarily belong to the Anisakidae family, consisting of two main genera: *Anisakis* and *Pseudoterranova*. The main objective of this study is to assess the occurrence of parasitism in fishery species along the Atlantic Ocean and Mediterranean Sea coasts in Morocco.

A total of 1808 specimens from 24 species were collected and examined for parasites between August 2022 and May 2023.

The results show that 279 samples were infested, giving an overall prevalence of 15.43%, which is very low; 3918 parasites were detected in 14 of the 24 species examined, giving an overall abundance of 2.17 and an intensity of 14.04. *Gymnorhynchus gigas* were exclusively found in the Atlantic Pomfret (*Brama brama*), exhibiting a high prevalence of 78.26%. In contrast, xenomas were observed in Axillary seabream (*Pagellus acarne*) (p=16.19%) and Atlantic horse mackerel (*Trachurus trachurus*) (p=1.01%). The parasites belonging to the Anisakidae family are the most widespread, with a percentage of 68.70%, and the silver scabbardfish (*Lepidopus caudatus*) has the highest prevalence, which is 87.14%. The examination of the correlation between its weight and the number of larvae yielded a correlation coefficient of r=0.52, while the correlation between its length and larvae count resulted in r=0.41. Both cases demonstrated a positive correlation.

These findings underscore the necessity of prioritizing the safety of fishery species to safeguard public health and ensure sustainable consumption practices.

Introduction

Due to its favorable geographical position, Morocco possesses two maritime fronts with approximately 3500 km of coastline. These advantages, coupled with a rich biological diversity, make the Moroccan coast one of the most abundant fishing areas, with an annual production potential of 1.556 million tons of fish, generating a turnover of 13.7 million MAD (DPM, 2022). As a result, Morocco stands as the leading fish producer in Africa and holds the 13th position among major fishing nations worldwide (FAO, 2024a). The fishing sector in Morocco plays a pivotal role in socio-economic development, contributing approximately 2% to the national gross domestic product. Beyond its vital role in ensuring food security (FAO, 2014), fisheries production significantly contributes to foreign trade (accounting for 8% of total exports and 33% of agri-food exports in 2020) and provides direct and indirect employment opportunities (DPM, 2022).

To date, the consumption of raw fish has been growing globally, but the elimination of the cooking step leads to emerging risks for consumers, like parasitic diseases. Moreover, parasitism remains one of the main reasons for the refusal to export these products from Morocco to the European Union (Dahani *et al.*, 2019), which can lead to loss of customers, imbalance in the exporter's budget, loss of jobs for employees, and damage to product image (Dahani *et al*., 2017).

Parasitism also poses significant health risks to humans, who become accidental hosts by ingesting L3 larvae of *Anisakis* spp., present in raw or undercooked fish flesh (Audicana and Kennedy, 2008). The most effective measure to eliminate these parasites in fishery products is freezing at -20°C for at least 24 hours (especially if it is intended for raw consumption), or processing through methods like cooking at over 60°C for 10 minutes or acidic marinating (Brutti *et al.*, 2010).

To control the risk associated with the presence of parasites, European regulation (EC No 2074/2005) requires visual inspection (European Commission, 2005): no fishery products obviously contaminated with visible parasites reach the consumers (Smaldone *et al.*, 2020).

In this context, the purpose of this study was to assess the occurrence of parasitism in 24 fishery species from various Moroccan Atlantic and Mediterranean harbors. In particular, the results will identify key species susceptible to infestation and the most prevalent parasites, contributing to a better understanding of the current parasitic phenomena in fishery products. Additionally, this research investigates the correlation between the weight and length of the species most likely to be infested and the number of parasites.

Materials and Methods

Sample collection

A total of 1808 fish specimens belonging to 24 species (Table 1) were sampled from various harbors along the Moroccan Atlantic (FAO Major Fishing Area 34.1) and Mediterranean (FAO Major Fishing Areas 37.1.1) coasts (FAO, 2024b).

The choice of the species was made based on the most caught and consumed in Morocco (FENIP, 2023).

Sample analysis

This work was carried out in the laboratory of the Food Safety Unit at the Hassan II Agronomic and Veterinary Institute, Rabat, Morocco.

Fish specimens' analysis

For fish specimens, the following parameters were measured:

- Total weight: corresponds to the weight in grams of the whole fish;
- Total length: in centimeters, measured from the most forward point of the head, with the mouth closed to the farthest tip of the tail with the tail compressed or squeezed, while the fish is lying on its side;
- Fork length: in centimeters measured from the most forward point of the head to the fork of the tail.

Parasites analysis

The inspection was conducted under good lighting conditions, using a magnifier when necessary.

For external parasites, a visual examination of the fish's exterior was performed to detect ectoparasites, which may be located on the skin, fins, gill chamber, and oral cavity.

For internal parasites, the search was carried out by making two incisions:

- First incision, from the anus to the bottom of the head, carefully avoiding tearing the viscera. This incision is made to search for parasites in the abdominal cavity.
- Second incision, made from the dorsal muscle towards the head, to search for parasites in the flesh.

Data analysis

The parasite population and infection density within the host population were quantified using the standard infection parameters established by Bush *et al.* [Eqs. 1-3]:

- Prevalence (%) (infested sample/sample) (P) $= \frac{\textbf{Number of sample infested (NSI)}}{\textbf{Number of sample examined (NSE)}} \times 100$ [Eq. 1]

- Abundance (parasites/sample) (A) $= \frac{\textbf{Number of parasites detected (NP)}}{\textbf{Number of sample examined (NSE)}}$ [Eq. 2]

- Intensity (parasites/infested sample) (I) $= \frac{\textbf{Number of parasites detected (NP)}}{\textbf{Number of sample infested(NSI)}}$ [Eq. 3]

The classification of the prevalence is based on the following categories and intervals: very high prevalence corresponds to a prevalence interval of 80-100%; high prevalence covers 60-80%; moderate prevalence includes 40-60%; low prevalence is defined as 20-40%; very low prevalence falls within 0-20%; and zero prevalence applies when the prevalence is 0%.

For the statistical analysis, SPSS software (IBM, Armonk, NY, USA) was used to perform a Wilcoxon signed-rank test to determine whether the distribution of xenomas and *Gymnorhynchus gigas* differed significantly between the left and right flanks of the fish. A two-tailed test with a significance level set at $p<0.05$ was applied. Microsoft Excel (Microsoft, Redmond, WA, USA) was employed to examine the correlation between the number of Anisakis larvae and the weight and length of the silver scabbardfish.

Results and Discussion

Overview of the parasitism phenomenon

Overall infestation parameters

In our study, we examined 1808 fish specimens from 24 species and detected parasitic infestations in 279 specimens belonging to 14 species. This corresponds to an overall prevalence of 15.43%, which is a very low prevalence. Comparatively, previous studies conducted in Morocco have reported markedly higher rates: Bouchriti *et al.* (2014) reported a prevalence of 47.7%, and Dahani *et al.* (2019) and Dahani *et al.* (2023) reported a prevalence of 31.4% and 20.77%, respectively.

The abundance of parasitic infestations in our study was 2.17, which is comparable to the abundance reported by Dahani *et al.* (2019) (2.23). However, it is considerably lower than the abundance reported by Bouchriti *et al.* (2014), which was 9.1, and the 6.51 reported by Dahani et *al.* (2023).

Regarding intensity, our study recorded a value of 14.04, which is higher than the 7.06 reported by Dahani *et al.* (2019) but considerably lower than the 31.38 reported by Dahani *et al.* (2023) and the 19 reported by Bouchriti *et al.* (2014).

These findings highlight that while our study shows a lower prevalence and abundance of parasitic infestations compared to previous national studies, the intensity of infestations remains relatively moderate. This demonstrates that while fewer fish are infested in our study, the number of parasites per infested fish is notable.

Types of parasites detected and their lesions

We detected parasites belonging to the following groups:

External parasites:

- Isopods (Figure 1a): these parasites often cause various pathologies in their hosts, such as epithelial erosions, inflammation and necrosis of the dermis developing at the point of attachment of the parasite to the skin, deformations of gill filaments and sometimes even significant mortality. Infested individuals become unfit for consumption (Khalifa, 2006).
- Leeches: the presence of leeches on fish gills can lead to significant health problems, including stress and susceptibility to secondary infections. In some cases, leech infestations can result in fungal infections due to wounds inflicted by the leeches (Burreson, 2006).

Internal parasites:

- Nematodes (Anisakidae) (Figure 1b): parasitized fish contain often tightly curled larval worms, most often found in the viscera. Affected areas may show slight inflammation, encapsulation, or granuloma formation. Visceral adhesion can occur in fish during heavy infestation. This leads to the production of fibrous connective tissue (Meyers *et al.*, 2019).
- Xeno-parasites (Figure 1c): when a microsporidian parasite infects a host cell, it takes control of its metabolism and proliferates within it. The parasite's mass then replaces most of the cell's cytoplasm. The host cell then envelops the proliferating parasite in layers of membranes and cells, forming a structure, called "xenoma". This structure provides the parasite with optimal growth conditions and protection from the host's immune response (Lom and Dyková, 2005).
- Cestodes (*Gymnorhynchus gigas*) (Figure 1d): this parasite can cause significant changes in musculature. The muscle tissue around the parasite may be less firm and yellowish compared to

the color of healthy muscle, histologically showing interstitial myositis. The parasite itself and these lesions are likely to reduce the duration of conservation of the fish (Panebianco, 1952).

The number of parasites found was 3918, of which 2751 were nematodes (70.21%), 1094 xeno-parasites (27.92%), 41 cestodes (1.05%), 30 isopods (0.77%) and two leeches (0.05%).

Location of parasites

External parasites

We found 32 external parasites (range 0–5 parasite/sample): two leeches in the gill chamber and 30 isopods, of which 10 are found in the oral cavity, 9 in the gill chamber, and 11 attached to the skin.

Although most marine isopods are benthic, some of them have evolved some swimming abilities and can be considered pelagic (Osborn, 2009), which explains the fact that isopods in our study are found in species from different habitats.

The overall prevalence of isopods is 1% which is in the interval of the results found by Ganapathy and Samuthirapandian (2013) along the Tamil Nadu coastal area in India, and lower than the study of Rania and Rehab (2015) in Egypt who found an overall prevalence of 4%.

As shown in Table 2, the annular sea bream occupies first place with a low prevalence of 33.33%, followed by striped seabream with a very low prevalence of 16.67%, then bogue (11.11%), silvery John dory (6.25%), striped red mullet 4.76%, Atlantic pomfret 4.35%, axillary seabream 2.82%, European hake 2,33%, John dory 1.92%, Rubberlip grunt 1.59%, mackerel 0.45% and finally horse mackerel 0.34%.

Internal parasites

A total of 3886 internal parasites were found, classified into three groups: nematodes (Anisakidae), cestodes (*Gymnorhynchus gigas*), and xeno-parasites.

Among nematodes (range: 0-116 larvae/sample), the majority (1798 larvae) were located in the viscera, followed by 641 in the abdominal cavity, 292 in the peritoneum, 21 in the gills, and none in the muscle (Table 3). Notably, a 7 cm long Anisakis was found in the gill chamber of a John Dory, and in one case involving a European hake, nematodes were observed in the gill chamber alongside a leech.

Xenoparasitic structures in the muscle (range: 0-260 xenomas/sample) showed varying distributions. In axillary seabream, 546 xenomas were identified on the right flank and 418 on the left flank, while in horse mackerel, 59 xenomas were found on the right flank and 71 on the left. For cestodes (range: 0-5 larvae/sample), 21 were observed on the right flanks and 20 on the left flanks of the Atlantic pomfret (Table 3). Statistical analysis revealed no significant differences in parasite distribution between the right and left flanks for xenomas ($p=0.121$) and *Gymnorhynchus gigas* ($p=1.000$).

Only one case of co-infection was recorded, involving an Atlantic horse mackerel infested by both nematodes and xenoparasites.

Specific infestation parameters

Analysis showed that 11.78% of the samples were infested by Anisakidae larvae L3. For comparison, studies have found a higher infestation rate in other regions, with 39.9% in Italy (Casti *et al.,*2017), 33.7% in Spain (Debenedetti *et al.*, 2019), and 33.58% in Southern Albania (Ozuni *et al.*, 2021). This comparison with international studies helps to provide a global perspective on the prevalence of Anisakidae larvae in fish.

In Morocco, the silver scabbardfish remains the most infested with this category of parasites and should therefore be systematically inspected for its presence.

This species has a very high prevalence of 87.14% (Table 4). This result is lower than the studies conducted by Bouchriti *et al*. (2014) and Dahani *et al.* (2023), who found a value of 100%. Next in line is the silvery John Dory, with a moderate prevalence of 56.25%. Then, the skipjack tuna with a low

prevalence of 39.29%. In the same category, we rank John Dory with a prevalence of 36.54%, followed by mackerel with a very low prevalence of 17.27%, Atlantic horse mackerel (10.85%), and European hake (9.30%).
Anisakidae larvae were found in different benthic and benthopelagic species. Its life cycle includes Crustacea as first, and larger invertebrates or fish as second intermediate hosts which means that its habitat varies that's why it shows little host specificity, as they've been reported from a wide variety of hosts (Klimpel *et al*., 2001; Klimpel *et al.*, 2006).
For *Gymnorhynchus gigas* (Table 4), we found a prevalence of 78.26%. This rate is lower than that reported by Dahani *et al*. in 2023 and Santoro *et al*. in 2022, both of which found a prevalence of 100%. In our study, as well as in the one conducted by Dahani *et al*. (2023), this cestode was exclusively found in the Atlantic pomfret. This differs from the findings of Dahani *et al*. (2019), where it was also observed in the silver scabbardfish (p=8.3%), and Giarratana *et al*. (2014) (p=72.31%).
All the above species are considered paranetic hosts for *Gymnorhynchus gigas* whose definitive hosts are Alopiidae (Pereira Bueno and Pérez, 1997).
In our study, these xenomas were present in the axillary seabream and the Atlantic horse mackerel, just like the study of Dahani *et al.* (2019), but in Dahani *et al.* (2023), it was found in the axillary seabream and the silver scabbardfish.
The infestation parameters of both species are mentioned in Table 4.

Correlation between weight, length and number of nematodes (Silver scabbardfish)
As the silver scabbardfish (*Lepidopus caudatus*) has the highest prevalence, a more in-depth study is conducted to analyze the correlation between the number of L3 larvae and both weight and length.
The analysis revealed a positive relationship between the total weight of fish and the number of larvae L3, as described by the regression equation (Figure 2).
The coefficient of determination (R^2=0.27) indicates that 27.47% of the variation in parasite count is explained by the weight of the fish.
The relationship between Silver scabbardfish's total length and the number of larvae L3 found was examined based on the correlation coefficient r=0.41, which reveals a positive correlation.
The coefficient of determination (R^2=0.17) means that the equation of the regression line determines 16.8% of the point distribution. It points to the quasi-absence of correlation.
The equation and coefficient of determination are presented in Figure 3.

Conclusions
This study highlights the presence of diverse groups of parasites in several fish species along the Moroccan coastline. Notably, the widespread occurrence of parasites, particularly Anisakids, raises major concerns due to their zoonotic potential, especially in the silver scabbardfish. In addition, other parasites identified in this study may negatively impact the organoleptic qualities leading to the rejection of the affected fish.
For the first time, this research identifies the occurrence of xeno-parasites in Atlantic horse mackerel and Anisakids in skipjack tuna in Morocco. These findings suggest an expanding range of host spectrums, now including additional fish species and emphasizing the need for enhanced monitoring and risk assessment.
This issue calls for effective management of parasitism, including prevention, control, and education. To address these challenges, collaboration among the fishing industry, regulators, and consumers is essential. Measures such as eviscerating fish at the point of capture and adhering to good hygiene practices in public establishments, including preventive freezing, should be implemented to reduce parasite prevalence.

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Table 1. Examined species and number of samples.

Family	Species	Scientific name	Habitat	N. of samples
Bramidae	Atlantic pomfret	*Brama brama*	Pelagic	23
Carangidae	Atlantic horse mackerel	*Trachurus trachurus*	Pelagic	295
Cepolidae	Red bandfish	*Cepola macrophthalma*	Benthic	21
Clupeidae	Sardine	*Sardina pilchardus*	Pelagic	383
Engraulidae	Anchovy	*Engraulis encrasicolus*	Pelagic	95
Haemulidae	Rubberlip grunt	*Plectorhinchus mediterraneus*	Demersal	63
Merlucciidae	European hake	*Merluccius merluccius*	Benthopelagic	258
Loliginidae	Squid	*Loligo vulgaris*	Benthopelagic	30
Mullidae	Striped red mullet	*Mullus barbatus*	Demersal	3
Scombridae	Mackerel	*Scomber scombrus*	Pelagic	220
	Skipjack tuna	*Katsuwonus pelamis*	Epi- or mesopelagic	28
Serranidae	Comber	*Serranus cabrilla*	Demersal	9
Sparidae	Axillary seabream	*Pagellus acarne*	Demersal	142
	Striped seabream	*Lithognathus mormyrus*	Demersal	6
	Bogue	*Boops boops*	Demersal and epiplagic	9
	Red porgy	*Pagrus pagrus*	Demersal	3
	Gilt-head sea bream	*Sparus aurata*	Demersal	3
	Salema porgy	*Sarpa salpa*	Demersal	6
	Sargo	*Diplodus sargus*	Demersal	9
	Annular sea bream	*Diplodus annularis*	Demersal	6
Trichiuridae	Silver scabbardfish	*Lepidopus caudatus*	Benthopelagic	70
	Largehead hairtail	*Trichiurus Lepturus*	Benthopelagic	6
Zeidae	John Dory	*Zeus faber*	Benthopelagic	104
	Silvery John Dory	*Zenopsis conchifer*	Benthopelagic	16

Table 2. Ectoparasite infestation parameters.

Species	NSE	NSI	N. of ectoparasites	Category by p	P (%)	A	I
Annular sea bream	6	2	2	Low prevalence	33.33	0.33	1.00
Striped seabream	6	1	1	Very low prevalence	16.67	0.17	1.00
Bogue	9	1	1		11.11	0.11	1.00
Silvery John dory	16	1	1		6.25	0.06	1.00
Red bandfish	21	1	3		4.76	0.14	3.00
Atlantic pomfret	23	1	1		4.35	0.04	1.00
Axillary seabream	142	4	7		2.82	0.05	1.75
European hake	258	6	7		2.33	0.03	1.17
John dory	104	2	2		1.92	0.02	1.00
Rubberlip grunt	63	1	5		1.59	0.08	5.00
Mackerel	220	1	1		0.45	0.00	1.00
Atlantic horse mackerel	295	1	1		0.34	0.00	1.00

NSE, number of sample examined; NSI, number of sample infested; P, prevalence; I, intensity; A, abundance.

Table 3. Location of internal parasites.

	Nematodes				Cestodes		Xeno-parasites		Total
	Abdominal cavity	**Gills**	**Peritoneum**	**Viscera**	**RF**	**LF****	**RF**	**LF**	
Silver scabbardfish	326	0	230	898	0	0	0	0	1454
Axillary seabream	0	0	0	0	0	0	546	418	964
Atlantic horse mackerel	92	0	33	251	0	0	59	71	506
John Dory	27	1	15	311	0	0	0	0	354
Mackerel	36	0	10	150	0	0	0	0	196
Silvery John Dory	115	0	0	45	0	0	0	0	160
Skipjack tuna	0	0	1	116	0	0	0	0	117
Atlantic pomfret	0	0	0	0	21	20	0	0	41
European hake	44	20	3	27	0	0	0	0	94
Total	640	21	292	1798	21	20	605	489	3886

RF, right flank; LF, left flank.

Table 4. Infestation parameters for each category of internal parasites.

Category	Species	NSE	NSI	N. of parasites	Category by p	p (%)	I	A
Anisakid	**Silver scabbardfish**	70	61	1454	Very high prevalence	87.14	23.84	19.92
	Silvery John dory	16	9	160	Moderate prevalence	56.25	17.78	10.00
	Skipjack tuna	28	11	117	Low prevalence	39.29	10.64	4.18
	John dory	104	38	354		36.54	9.32	3.40
	Mackerel	220	38	196	Very low prevalence	17.27	5.16	0.89
	Atlantic horse mackerel	295	32	376		10.85	11.75	1.27
	European hake	258	24	94		9.30	3.92	0.36
Gymnorhynchus gigas	**Atlantic pomfret**	23	18	41	High prevalence	78.26	2.28	1.78
Xenomas	**Axillary seabream**	142	23	964	Very low prevalence	16.19	41.91	6.79
	Atlantic horse mackerel	295	3	130		1.01	43.33	0.44

NSE, number of sample examined; NSI, number of sample infested; P, prevalence; I, intensity; A, abundance.

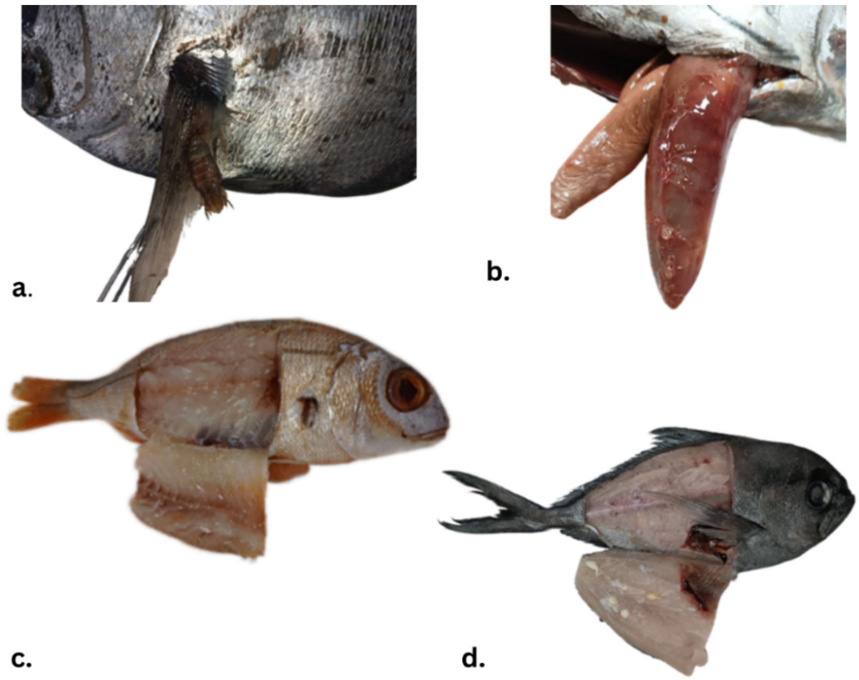

Figure 1. a) Isopod attached to the skin behind the pectoral fin of a John Dory; b) infestation of Skipjack tuna by Anisakdiae's L3; c) infestation of Axillary seabream by xeno-parasites; d) infestation of Atlantic pomfret by *Gymnorhynchus gigas*.

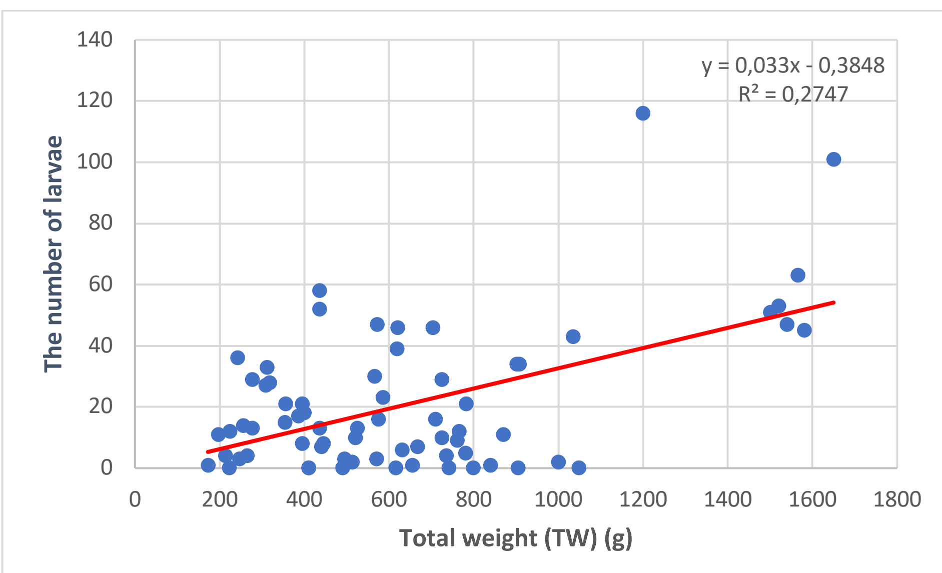

Figure 2. Trend line between total weight and number of larvae.

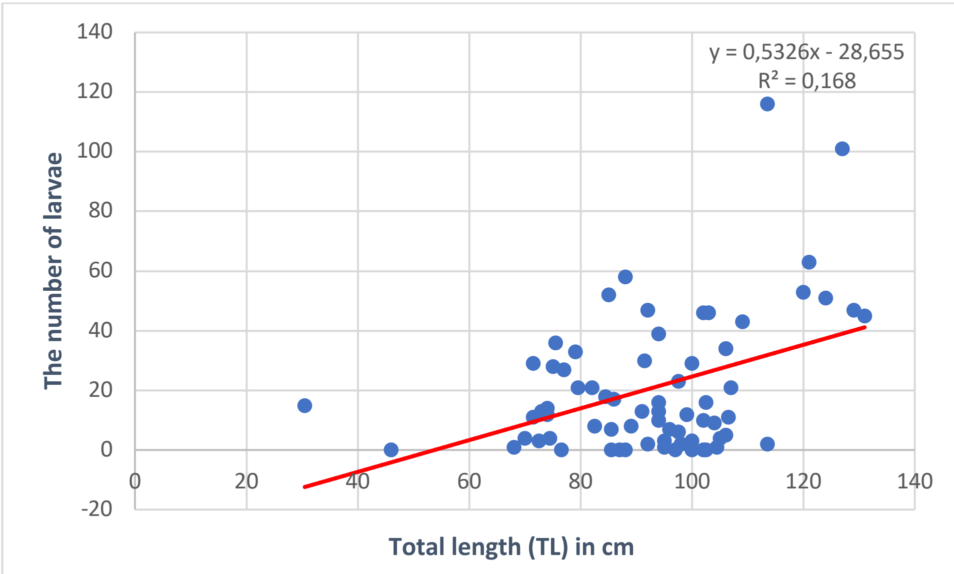

Figure 3. Trend line between total length and number of larvae.