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Malacologie

THE CONDITION INDEX: AN EFFECTIVE ECO-PHYSIOLOGICAL INDICATOR IN THE INVASIVE COCKLE *FULVIA FRAGILIS*

par

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This study is the first on condition index monitoring for *Fulvia fragilis,* a non-indigenous and invasive species in Tunisia. It is a simultaneous hermaphroditic cockle with a diffuse gonad. Condition indices (total and those relating to the visceral mass (gonad and digestive gland) and the rest of the flesh) were monitored monthly in two northern Tunisian sites (Bay of Tunis and lagoon of Bizerte). In favourable conditions, as found in the Bay of Tunis, the condition index reflects the progress of gametogenesis with synchronous increases in maturation and spawning periods. In the Bay of Tunis population, the strong correlation between the total condition index (CI) and partial spawning stage reflects low gamete emission. This index can be used as an eco-physiological index, since it reflects well the deterioration of the cockle metabolism in polluted environments, such as Bizerte lagoon. In addition, seasonal and synchronous development of the visceral mass condition index (CI_{VM}) and the remaining tissues condition index (CI_R) indicates simultaneous gonadic and somatic development in favourable conditions (nutrient availability and temperatures) in this non indigenous cockle.

Keywords: *Fulvia fragilis,* invasive species, condition index, visceral mass condition index, remaining tissues condition index, reproduction, environmental parameters, pollution.

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Indice de condition : un indicateur éco-physiologique effectif chez la coque invasive *Fulvia fragilis*

Cette étude constitue le premier travail portant sur le suivi de l'indice de condition chez la coque non indigène invasive *Fulvia fragilis* (Forsskål in Niebuhr, 1775). Cet indice est un indicateur de stress, de l'activité sexuelle et de l'état physiologique de l'animal et permet de suivre les différentes variations de la balance métabolique des individus à travers les changements saisonniers sous l'influence des facteurs internes et externes. Dans ce travail, nous avons jugé utile de séparer la masse viscérale (gonade, glande digestive et pied) du reste de la chair (muscles adducteurs, manteau, branchies…) dans le but d'affiner nos résultats et de calculer ceux relatifs à la masse viscérale (CI_{VM}) et au reste de la chair (CI_R) séparément en plus de l'indice de condition total (CI). Ainsi, un suivi mensuel des indices de conditions, dans le nord de la Tunisie (La baie de Tunis et la lagune de Bizerte) a été effectué. Étant donné que *F. fragilis* présente une gonade diffuse entre la glande digestive et la construction pédieuse, l'indice de condition de la masse viscérale ne constitue pas un indice gonado-somatique ; cependant, il donne une idée sur les changements physiologiques liés au cycle de la reproduction. En effet, sa gonade est rétractée durant les périodes de repos sexuel et devient volumineuse durant la période d'activité sexuelle. En outre, l'indice de condition du reste des chairs ne constitue pas un indice propre aux tissus somatiques. Toutefois, il renseigne sur les périodes d'amaigrissement et de stockage des réserves nutritives. Notre étude stipule que l'indice de condition est un bon indicateur de l'état physiologique chez la coque invasive *F. fragilis*. Dans un milieu favorable à la reproduction, comme la baie de Tunis, il reflète bien le déroulement de la gamétogenèse avec des hausses synchrones avec les périodes de production gamétique (maturation et ponte partielle). Dans un milieu pollué et défavorable à la reproduction, le cas de la lagune de Bizerte, cet indice peut être utilisé comme un indice écophysiologique reflétant bien la détérioration du métabolisme de l'animal. La forte corrélation entre l'CIMV et le stade « ponte partielle », dans la population de *F. fragilis* de la baie de Tunis, traduit une faible émission gamétique expliquant la ponte étalée dans le temps chez cette espèce. Par ailleurs, l'évolution saisonnière et synchrone des deux indices, à savoir l' CI_{VM} et l'CI_R, stipule un développement gonadique et un accroissement en chair simultanés, en conditions favorables (disponibilité en nutriments et températures favorables).

Mots-clés : *Fulvia fragilis,* espèce invasive, Indice de condition, Indice de condition de la masse viscérale, Indice de condition du reste, reproduction, paramètres environnementaux, pollution.

Introduction

The privileged geographical position of Tunisia in the Mediterranean, between the western and eastern basins, explains its remarkable biodiversity. *Fulvia fragilis* (Forsskål in Niehbur, 1775), an introduced and invasive species in Tunisian coastal waters, was first reported in 1996 in the Gulf of Gabès (PASSAMONTI, 1996) and has expanded its range to the northern coasts (BEN SOUISSI, 2003; ZAOUALI, 2004). In previous studies (RIFI *et al.,* 2011; RIFI *et al.,* 2012), we used qualitative (histological analysis) and quantitative (oocyte diameter measurements) methods to survey the reproduction of this species in the Bay of Tunis. In the present work, the condition index was followed at two sites on the Northern Tunisian coast, namely the

Bay of Tunis and the Bizerte Lagoon. The condition index is a quantitative method used to provide information on the physiological state and sexual activity of molluscs (LUCAS & BENINGER, 1985). The condition index was shown to be an effective measure in several species of bivalve mollusks with dissociate gonad, such as the oyster *Crassostrea gigas* (ENRIQUEZ-DIAZ, 2004), the mussels *Mytilus edulis* (BRESSAN & MARIN, 1985) and *Mytilus galloprovincialis* (YILDIZ *et al.,* 2006) and in other species with diffuse gonad, such as the clams *Ruditapes decussatus* and *Ruditapes phillipinarum* (LARUELLE, 1999) and the cockle *Cerastoderma edule* (GUILLOU *et al.,* 1992). Considering that the gonad of *F. fragilis* is diffuse and extends between the digestive gland and the pedal constriction, we separated the visceral mass (gonad, foot and digestive gland) from the rest of the flesh and we calculated the visceral mass condition index (CI_{VM}) and remaining tissues condition index (CI_R) in order to refine our results. Thus the somatic and reproductive energies were assessed separately. Condition indices were correlated with environmental parameters and histological observations and compared for each geographical site. This study aims to compare the physiological and reproductive activities of the invasive cockle *F. fragilis* in marine and lagoon waters, especially the Bay of Tunis and Bizerte Lagoon in northern Tunisia. These two sites were selected because the species was present abundantly throughout the year, thus allowing regular biological monitoring.

Material and methods

Sampling sites

The Bay of Tunis, also known as 'small Gulf of Tunis', is located in the south-east of the Gulf of Tunis (Fig. 1). Our sampling site is situated at latitude 36°42' and longitude 10°11'. The Gulf of Tunis represents the southern limit of the Siculo-Tunisian channel, which is a ship transit line, constituting hydrological, climatic and geographical borders between the east and the west of the Mediterranean basin (PERES, 1967). This bay is a relatively sheltered zone (BEN CHARRADA & MOUSSA, 1997; BEN CHARRADA *et al.,* 1997), located near the two most important ports in Tunisia, namely Radès and La Goulette. The biodiversity of the Bay has been studied and the general environmental status is considered satisfactory (AYARI & AFLI, 2003).

Bizerte Lagoon is located in the north of Tunisia between latitudes 37°8'-37°14' and longitudes 9°46'-9°56'. It is an elliptical depression of 128 km^2 (Fig. 1), making it the largest lagoon area of northern Tunisia. It communicates with the Gulf of Bizerte through a channel and is connected to Ichkeul Lake (the largest lake in Tunisia) via the Tinja Oued (BELKHODJA *et al.,* 2007; BEN GARALI *et al.,* 2011). This lagoon is heavily polluted because of several anthropogenic factors (port, aquaculture, urban development, agriculture, industry, etc.). Previous studies have demonstrated that this site is polluted with organics and metals and shows disturbances in its fauna and flora (AISSA, 1991) as well as in its biochemical and geochemical cycles (YOSHIDA *et al.,* 2002a, 2002b, 2003; BENGARALI *et al.,* 2009, 2010).

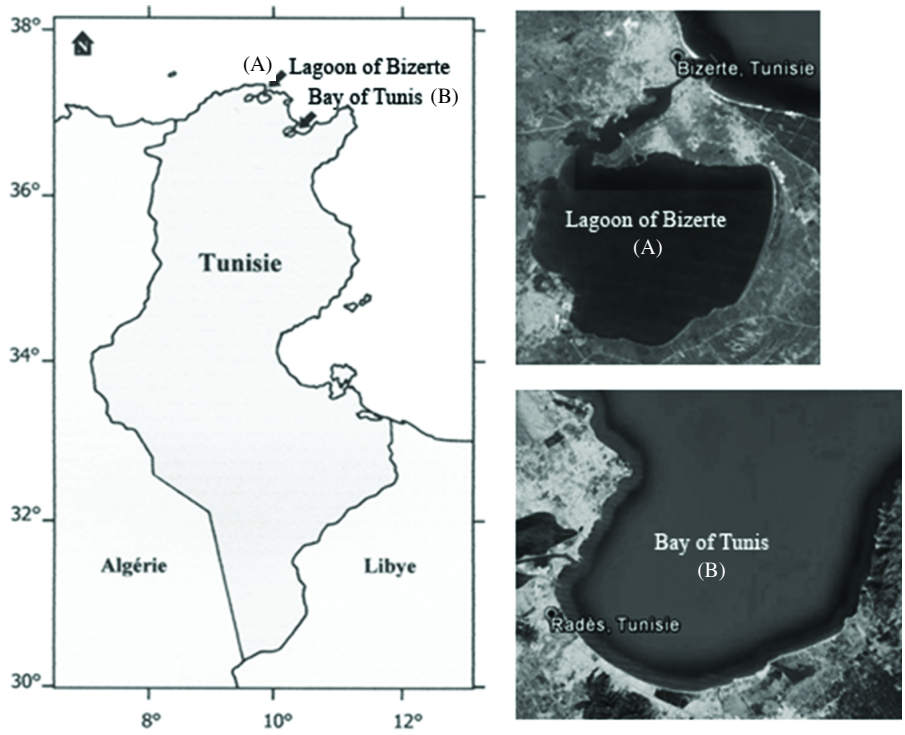

Figure 1

Localization of sampling sites (Lagoon of Bizerte (A) and Bay of Tunis (B)).
Localisation des sites d'échantillonnage (Lagune de Bizerte (A) et Baie de Tunis (B)).

Sampling

Specimens of *Fulvia fragilis* were collected monthly from January 2006 to October 2007 in the Bay of Tunis and from June 2006 to September 2007 in Bizerte Lagoon. Sampling was not carried out in the Bay of Tunis during July, August and September 2006 because of high summer mortalities of this non indigenous cockle. In the Bay of Tunis, a total of 3450 specimens of *F. fragilis* were collected at very shallow depths (0.5-1.5 m), in sandy-mud sediment using an experimental drag and SCUBA diving. In Bizerte Lagoon, 1869 specimens were collected in muddy sediment, at 5 m depth using an experimental drag fired from a motorboat. Monthly totals of specimens were used for condition index monitoring and histological analysis with corresponding mean monthly shell length, as detailed in tables 1 and 2.

Environmental parameters

The temperature of the sea surface was taken using a thermometer with an accuracy of ± 0.1°C. A WTW 340i multi-parameter meter was used for the measurements of pH, salinity and dissolved oxygen. The chlorophyll-a concentration was

Table 1

Monthly numbers of specimens used for condition index monitoring and histological analysis with corresponding mean monthly shell length ± SD (Bay of Tunis, January 2006-October 2007).

Effectifs mensuels des spécimens utilisés pour le suivi de l'indice de condition et de l'histologie et longueurs moyennes mensuelles ± Écart type (Baie de Tunis, Janvier 2006-Octobre 2007).

Year	Month	CI monitoring effective	Shell length ±SD (mm)	Histology analysis effective	Shell length ± SD (mm)
2006	Jan	168	36,84±3,88	-	
	Feb	168	38,85 ±4,01	-	
	Mar	100	39,48±4,26	-	
	Apr	117	41,27±3,89	30	39,85±3,70
	May	91	35,16±6,37	30	39,7±5,37
	Jun	93	41,08±5,21	30	43,45±4,61
	Oct	129	26,12±5,06	30	33,43±2,07
	Nov	134	33,62±5,09	30	36,7±1,99
	Dec	180	33,8±3,59	30	40,40±3,30
2007	Jan	201	34,59±3,46	30	37,59±3,44
	Feb	146	37,28±5,12	30	36,83±4,20
	Mar	223	35,62±3,5	30	37,47±2,63
	Apr	252	39,46±4,36	30	39,22±3,47
	May	519	39,26±3,73	60	38,48±3,69
	Jun	278	41,56±3,94	60	38,45±3,86
	Jul	94	40,6±6,91	60	43,03±3,01
	Aug	157	42,67±3,11	30	48,65±2,45
	Sept	66	43,45±3,49	7	43,71±4,70
	Oct	27	43,45±3,58	30	44,28±3,27

measured according to the method described by AMINOT & CHAUSSEPIED (1983).

Histology

A total of 228 specimens, collected from Bizerte Lagoon in July and August 2006 and between April and September 2007, was used for the histological reproduction study. The visceral mass was dissected, fixed in Bouin's solution for 48 hours and stained with haematoxylin–eosin according to the method described by GABE (1968). The stage of gametogenesis was determined according to RIFI *et al*. (2011). Male and female germinal cells, evolving simultaneously, were taken into account to determine the gametogenesis stage. The evolution of the gametogenesis in *F. fragilis* in Bizerte Lagoon was compared to that described in the Bay of Tunis in RIFI *et al*. (2011).

Table 2

Monthly numbers of specimens used for condition index monitoring and histological analysis with corresponding mean monthly shell length ± SD (Bizerte Lagoon, June 2006-September 2007).

Effectifs mensuels des spécimens utilisés pour le suivi de l'indice de condition et de l'histologie (Lagune de Bizerte, Juin 2006-Septembre 2007).

Year	Month	CI monitoring effective	Shell length ±SD (mm)	Histology analysis effective	Shell length ± SD (mm)
2006	Jun	190	29±0,24	-	-
	July	177	31,07±0,25	30	32,99±2,26
	Aug	104	32,66±0,32	30	38,52±2,43
	Sept	165	36,03±0,25	-	-
	Oct	94	37,40±0,34	-	-
	Nov	123	38,30±0,29	-	-
	Dec	109	36,50±0,31	-	-
2007	Jan	123	38,30±0,29	-	-
	Feb	86	36,32±0,35	-	-
	Mar	108	38,88±0,31	-	-
	Apr	126	36,81±0,29		
	May	173	37,56±0,43	30	40,89±0,52
	Jun	143	38,00±0,43	30	39,92±0,99
	Jul	74	44,78±0,34	30	41,66±2,92
	Aug	21	41,79±0,46	10	39,83±3,07
	Sept	15	43,27±0,54	8	40,54±2,55

Condition Index

There are numerous methods in the literature for calculating the condition index; some formulas use fresh weight and others dry weight. Using fresh tissue weight may bias the results because of water fluctuations in bivalve tissues (LUCAS & BENINGER, 1985). Accordingly, we decided to use the dry tissue weight in our analyses.

To obtain the dry weight we used oven drying. Tissues of *Fulvia fragilis* were dried in an oven at 60°C for 72 hours. From October 2006 onwards, the visceral mass (digestive gland, gonad and foot) was separated from the rest of the soft tissues, at both sites, in order to refine the results, and the visceral mass condition index and rest condition index were calculated. The dry shell weight was determined for each animal.

Condition indices were calculated using the following formulas:

– CI_{Tot} = (DFW /DshellW)x100 (MANN & GLOMB, 1978)

– CI_{VM} = (DVMW /DshellW)x100

– CI_{R} = (DRW/DshellW)x100

where CI_{Tot} is Total Condition Index; CI_{VM} is Visceral Mass Condition Index; CI_{R} is Remaining soft tissue Condition Index; DFW is Dry Flesh Weight; DVMW is Dry Visceral Mass Weight; DRW is Dry Rest Weight and DshellW is Dry shell Weight.

Data analysis

The condition indices were correlated with the physiochemical parameters (Temperature, Chlorophyll-a concentration, dissolved oxygen concentration, salinity and pH) using principal component analysis. Correlations between condition indices and gametogenesis stages were evaluated using the Pearson Product correlation. All data analyses were carried out with the Statgraphics Centurion software.

Results

Environmental parameters

Temperature and chlorophyll-a concentration

In the Bay of Tunis, the average water temperature was 23.8±5.8°C (Fig. 2). Temperature values ranged between 12.4°C (February 2006) and 30.5°C (August 2006). Spring temperatures varied between 16 and 23°C. In Bizerte Lagoon, the average temperature was 22.9 ± 5.7°C. Seasonal changes were noted, with the lowest values being recorded in January (11.1°C) and February (13.7°C) of 2007. The maximum temperatures, exceeding 28°C, were recorded in August 2006 and 2007. This high temperature persisted until September in 2007 (28.8°C). The spring water temperatures ranged between 15 and 23.5°C (Fig. 2).

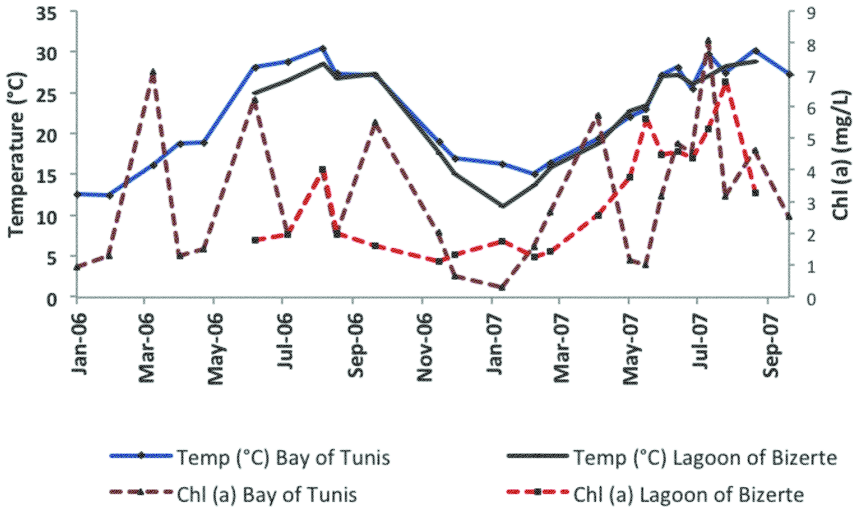

Figure 2

Monthly variations in sea surface temperature (°C) and Chlorophyll-a concentration (mg/L) in the Bay of Tunis and in Bizerte Lagoon.

Variations mensuelles de la température de l'eau et de la concentration de la chlorophylle a dans la Baie de Tunis et dans la lagune de Bizerte.

Chlorophyll-a concentration varies seasonally at the two sites. In the Bay of Tunis, the average concentration was 3.03 ± 2.22 mg/L (Fig. 2). The lowest concentrations were recorded in winter, with a minimum level of 0.28 mg/L in January 2007. During the rest of the year we noted seasonal peaks, the most important of which were observed during the spring and summer periods, including March 2006 (7.08 mg/L) and July 2007 (8.09 mg/L). In Bizerte Lagoon, the average chlorophyll-a concentration was 3.12 ± 1.73 mg/L. The highest values were recorded in spring and summer, with a maximum value of 7.77 mg/L in August 2007, and the lowest value was noted in winter in November 2006 (1.11 mg/L) (Fig. 2).

Salinity and pH

The average salinity of the small Gulf of Tunis was 36.4 ± 1.6 psu (Fig. 3). The lowest values were observed in winter, with a minimum of 34.8 psu in February 2006. The highest values were observed in hot months, with a maximum of 38.5 psu in August 2007. In Bizerte Lagoon, the average salinity was 36.00 ± 0.98 psu. Changes in this parameter are seasonal and the lowest values are observed in the colder months. These fluctuations were due to heavy rains and fresh water inflow from neighbouring lakes such as those recorded in February 2007 (34.07 psu). The highest values were recorded in June 2007 (37.80 psu) (Fig. 3).

In the Bay of Tunis, the recorded pH values showed small fluctuations (8.20 ± 0.08), indicating no significant change in the chemical composition of the water's surface (Fig. 3). The extreme value was recorded in January 2007 (8.34). The average

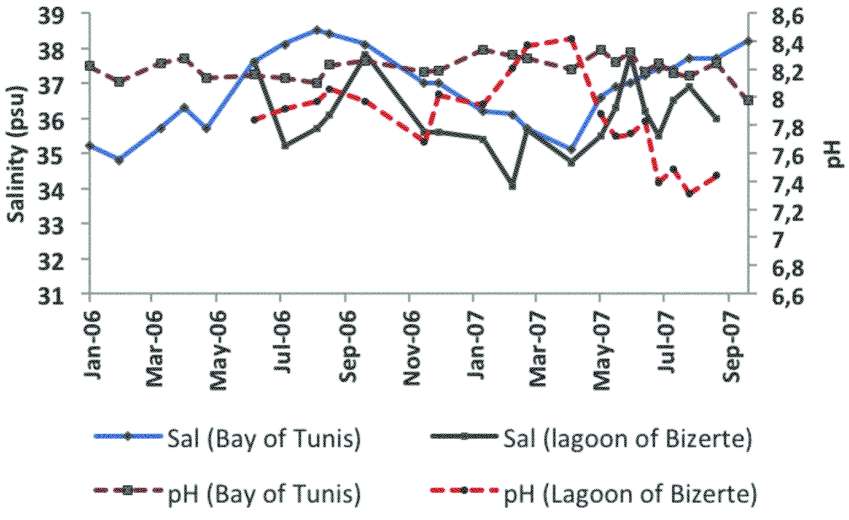

Figure 3

Monthly variations in salinity (psu) and pH in the Bay of Tunis and in Bizerte Lagoon.
Variations mensuelles de la salinité et du pH dans la Baie de Tunis et dans la lagune de Bizerte.

pH recorded in Bizerte Lagoon was 7.85, and it varied between 7.31 (August 2007) and 8.42 (April 2007) (Fig. 3). Compared to the Bay of Tunis, this parameter showed marked fluctuations. pH is a reliable indicator of water quality. The pH variability observed in Bizerte Lagoon is probably due to pollution.

Dissolved oxygen concentration

In the Bay of Tunis, surface waters were under-saturated with dissolved oxygen during the summer months. Throughout the rest of the year there was adequate oxygenation with an average level of 5.75 mg/L. High levels were recorded in winter and spring 2007. The average value of the dissolved oxygen concentration recorded in the Bizerte lagoon was 4.46 mg/L, with a maximum value of 7.60 mg/L (December 2006) and a minimum value of 2.58 mg/L (May 2007) (Fig. 4).

Temporal evolution of gametogenetic stages

The histology of *F. fragilis* gametogenesis was detailed in a previous work (RIFI *et al*., 2011). This invasive cockle is a simultaneous hermaphroditic species, having a non-dissociate gonad extending between the digestive gland and the foot. In this paper, we briefly outline the temporal distribution of gametogenesis at the two study sites. In the Bay of Tunis, *F. fragilis* showed continuous sexual activity with permanent spawning throughout the year (Fig. 5). Our study revealed that *F. fragilis* presents an asynchronous gametogenetic cycle between individuals and between fol-

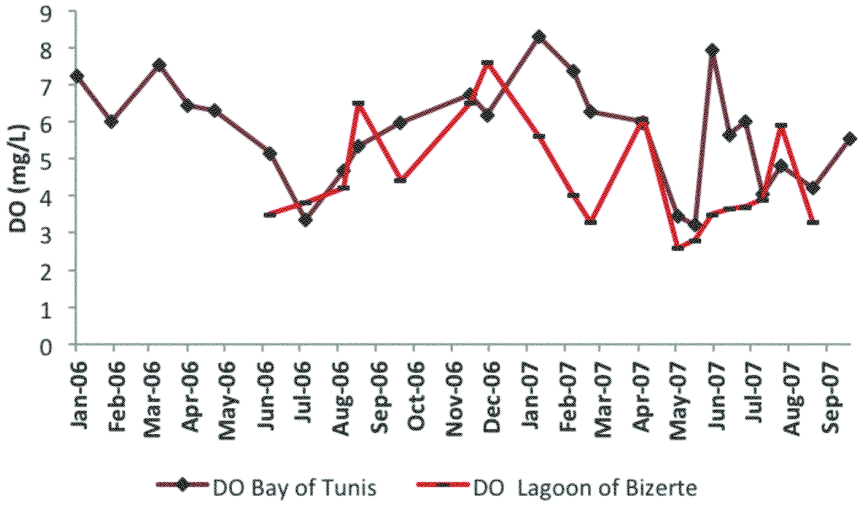

Figure 4

Monthly variations in dissolved oxygen concentration (mg/L) in the Bay of Tunis and in Bizerte Lagoon.

Variations mensuelles de la concentration en oxygène dissous (mg/L) dans la Baie de Tunis et dans la lagune de Bizerte.

licles of the same individual; this phenomenon is common in bivalves of temperate regions (RIFI *et al*., 2011). In contrast, in Bizerte Lagoon, a low sexual activity was observed, most likely due to the high environmental pollution (Fig. 6). Indeed,

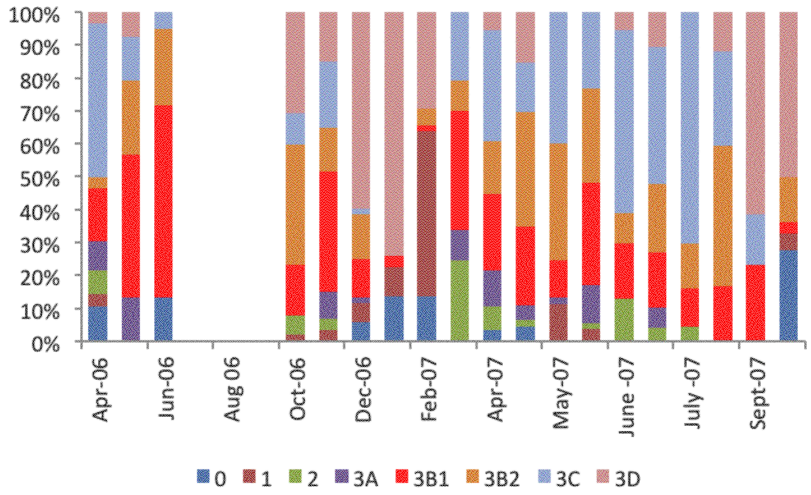

Figure 5

Temporal distribution of *F. fragilis* gametogenetic stages in the Bay of Tunis (April 2006-October 2007, from RIFI et al., 2011).

Distribution temporelle des stades gamétogéniques chez F. fragilis *dans la Baie de Tunis (Avril 2006-Octobre 2007 in RIFIet al., 2011).*

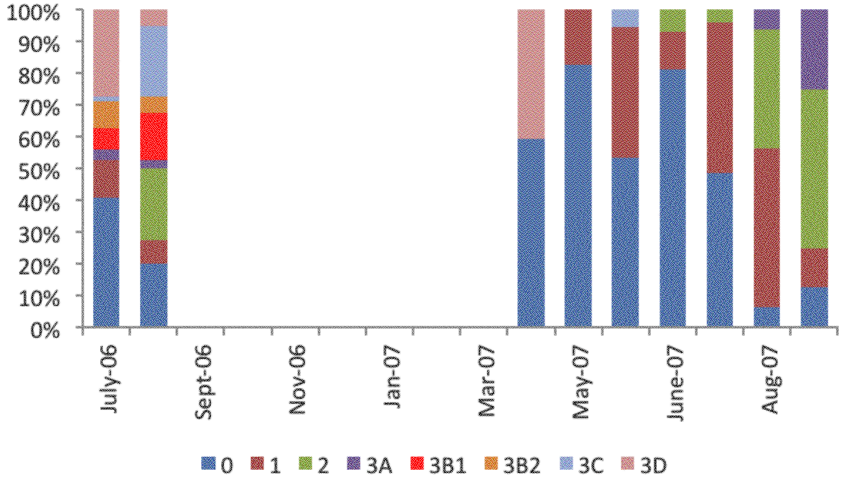

Figure 6

Temporal distribution of *F. fragilis* gametogenetic stages in Bizerte Lagoon (July and August 2006 and April 2007-September 2007).

Distribution temporelle des stades gamétogéniques chez F. fragilis *dans la lagune de Bizerte (Juillet et Août 2006 et Avril-Septembre 2007).*

among the specimens examined in Bizerte Lagoon, the predominant gametogenetic stage, between April and September 2007, was that of sexual rest, in contrast to the cockles collected in the Bay of Tunis (Figures 5 and 6). Although castration parasites can inhibit reproduction in many other bivalve species (VALDERRAMA *et al.*, 2004; GURNEY *et al.*, 2004; NGO *et al.*, 2006), no such parasites were found in the material examined, hence this possibility can be excluded.

Condition index

To better understand the sexual cycle of *F. fragilis*, the condition index was monitored at the two study sites. Although the visceral mass condition index is not a gonadosomatic index, it does reflect gonadic physiological changes. Indeed, the gonad decreases in size during sexual rest and increases in volume during periods of sexual activity. In addition, the index of the remaining tissue was not correlated with the somatic tissue index, but it did reflect periods of weight loss and nutrient reserves storage.

Temporal condition index evolution in the Bay of Tunis

In the Bay of Tunis, the total *F. fragilis* condition index shows seasonal variations. Successive summer peaks were recorded in June 2006 (13.51 ± 5.05) and June 2007 (15.10 ± 5.78), autumnal increases in October 2006 (14.76 ± 4.06) and November 2006 (14.86 ± 5.17) and the highest spring values were noted in April 2007 (15.29 ± 3.77) (Fig. 7). A winter decrease was recorded in January 2007 (7.61 ± 1.06). The three

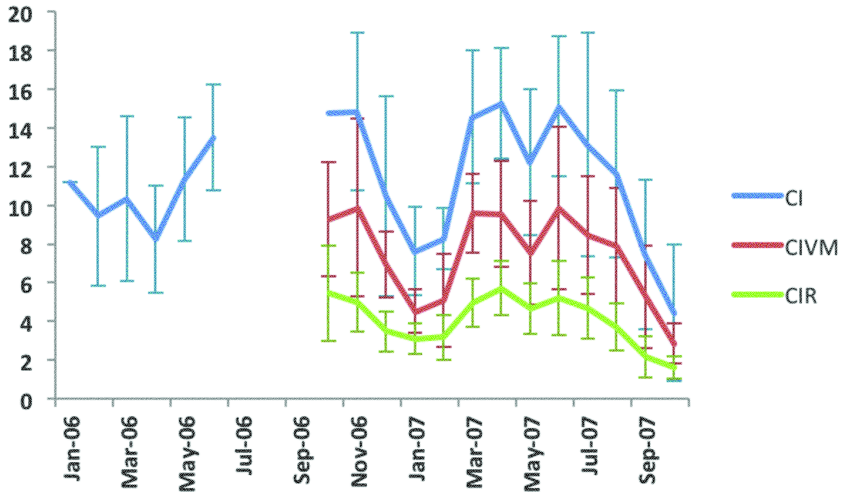

Figure 7

Temporal condition indices (CI_{Tot}, CI_{VM}, CI_R) in the Bay of Tunis (January 2006-October 2007).

Évolution temporelle des indices de condition (CI_{Tot}, CI_{VM}, CI_R) dans la Baie de Tunis (Janvier 2006-Octobre 2007).

indices, namely CI_{Tot}, CI_{VM} and CI_R, followed the same trend in the period October 2006-October 2007. We noted that CI_{VM} was always greater than CI_R.

The monthly CI_{Tot} standard deviations were relatively high (1.51 to 5.78), mainly due to the monthly CI_{VM} standard deviation (1.04 to 4.60). The corresponding CIR standard deviations were lower. These observations reflected an intra-populational asynchrony, essentially explained by physiological changes in the visceral mass and, especially, the gonad. These results were similar to those demonstrated in our histological study.

Correlations between condition index and environmental parameters in the bay of Tunis

A Principal Component Analysis (PCA) was applied in order to determine whether any correlation existed between the total condition index and measured environmental parameters of the two sampling sites.

In the Bay of Tunis, two principal components explaining 65.70 % of the total inertia were retained (Fig. 8). Thus, the *F. fragilis* CI showed a strong positive correlation with the chlorophyll-a concentration, a positively correlated with temperature and negative correlations with pH and dissolved oxygen concentration. Summer, autumnal and spring CI increases coincided with chlorophyll-a concentration peaks, high temperatures and low dissolved oxygen concentrations. Thus, increases in CI were noted in June 2006, October and April 2006, when the chlorophyll-a concentrations exceeded 5 μg/L. In contrast, the winter CI decrease, in

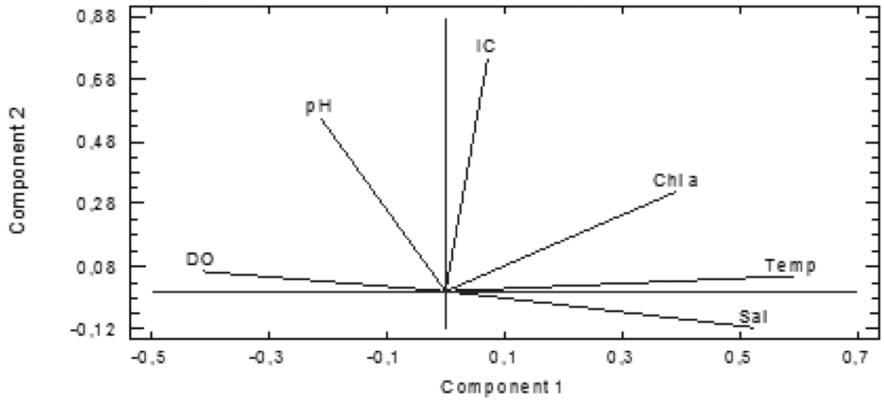

Figure 8

Principal component analysis (PCA) between environmental parameters and total Condition Index in the Bay of Tunis.
CI, Total condition index; Chl a, chlorophyll-a concentration; DO, dissolved oxygen; Sal, salinity; Temp, sea surface temperature.

Analyse en composantes principales (ACP) entre les paramètres environnementaux et l'Indice de Condition dans la Baie de Tunis.

Table 3

Pearson product moment analysis between Condition indices (CI_{Tot}, CI_{VM}, CI_R) and gametogenesis stages [**r**: correlation coefficient, **P**: probability (significant if P< 0.05)].

*Corrélations de Pearson Product-moment entre les IC (IC total, IC Mv et IC Reste) et les stades gamétogénétiques [**r** : coefficient de corrélation, **P** : probabilité (significatif quand P< 0,05)].*

Indices	Parameters	Stage 0	Stage 1	Stage 2	Stage 3A	Stage 3B1	Stage 3B2	Stage 3C	Stage 3D
CI_{Tot}	**r**	-0,67	-0,34	-0,32	**0,47**	**0,53**	**0,55**	0,26	-0,65
	P	0,0016	0,1504	0,1726	0,0392	0,0175	0,0146	0,2789	0,0024
CI_{VM}	**r**	-0,78	-0,38	0,39	**0,69**	**0,71**	**0,50**	0,40	-0,70
	P	0,0004	0,1360	0,1291	0,0031	0,0017	0,0465	0,1224	0,0022
CI_R	**r**	-0,64	-0,26	0,37	**0,67**	**0,56**	**0,53**	0,32	-0,68
	P	0,0074	0,3297	0,1558	0,0040	0,0221	0,0345	0,2134	0,0035

r: correlation coefficient, **P**: probability (significant if P< 0,05).

January 2007, coincided with the minimum value of the chlorophyll-a concentration (0.65 mg/L). This period corresponds with low temperatures and high dissolved oxygen concentration.

Correlation between condition indices and gametogenesis stages in the bay of Tunis

To evaluate the significance of condition indices in *F. fragilis,* we used the Pearson Product method to assess their correlation with different sexual maturation stages (Table 3). All condition indices (CI_{Tot}, CI_{VM} and CI_R) were negatively correlated with the total spawning stage (3D) (Table 3). The CI_{Tot} was positively correlated with the maturation stage and strongly positively correlated with advanced spawning and partial spawning stages (r>0.5). The CI_{VM} was strongly correlated with maturation and partial spawning stages and positively correlated with advanced spawning stage. The CI_R was strongly correlated with maturation and partial spawning stages. Therefore, in this invasive bivalve, all indices are correlated with gamete production. Moreover, the changes in the condition indices (CI_{Tot}, CI_{VM} and CI_R) for *F. fragilis* in the Bay of Tunis were generally synchronous with the spawning periods. Their autumn, spring and summer increases coincided with spawning peaks and their decreases (January 2007-October 2007) coincided with periods of low gonadic activity.

Temporal condition index evolution in Bizerte Lagoon

In Bizerte Lagoon, average monthly *F. fragilis* CI_{Tot} values showed relatively high values during the first five months of the survey, with a peak recorded in June 2006 (12.34±2.97) (Fig. 9). From November 2006 until the end of the sampling period, the CI_{Tot} showed a significant decrease, with a minimum in July 2007 (6.15±2.55). These low values of CI reflect poor physiological conditions of the cockle.

The CI_{VM} and CI_R values developed differently. Both indices showed low values throughout the study period, but in October 2006, the CI_{VM} was always greater than the CI_R (Fig. 9). The CI_{VM} average values varied between 2.32±0.97 (October 2006) and 5.15±1.20 (August 2007) (Fig. 9). These values reflected a low gonadic

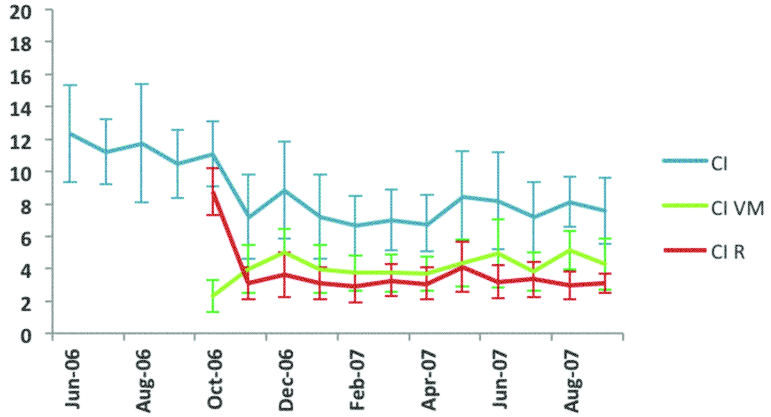

Figure 9

Temporal condition indices (CI_{Tot}, CI_{VM}, CI_R) in Bizerte Lagoon (June 2006-September 2007).

Évolution temporelle des indices de condition (CI_{Tot}, CI_{VM}, CI_R) dans la lagune de Bizerte (Juin 2006-Septembre 2007).

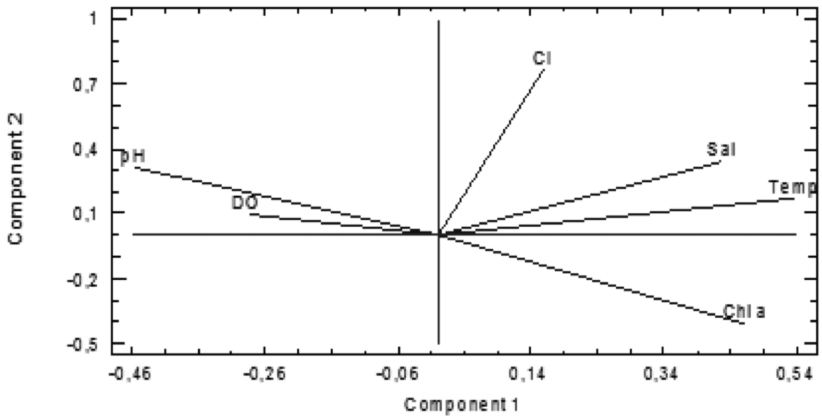

Figure 10

Principal component analysis (PCA) between the environmental parameters and Condition index in Bizerte lagoon.

CI, condition index; **Chl a**, chlorophyll-a concentration ; **DO**, dissolved oxygen; **Sal**, salinity; **Temp**, sea surface temperature.

Analyse en composantes principales (ACP) entre les paramètres environnementaux et l'Indice de Condition dans la lagune de Bizerte.

activity, confirmed by histological gonadic tissue observations, during the period of April to September 2007. The CI_R also showed low values, except in October 2006 (8.77±1.44). These observations reflect reproductive abnormalities in the population of *F. fragilis* in Bizerte Lagoon.

Correlations between the condition index and environmental parameters in Bizerte Lagoon

In order to determine possible correlations between the condition index and environmental parameters, we carried out a principal component analysis. Two principal components, explaining 68.90% of the total inertia, were retained. Thus, the CI for *F. fragilis* in Bizerte lagoon was positively correlated with water surface temperature and salinity, very weakly correlated with chlorophyll-a concentration and negatively correlated with pH and dissolved oxygen (Fig. 10).

Discussion and conclusion

The condition index is a physiological index reflecting changes in body tissues and the periodicity of the sexual cycle (LUCAS & BENINGER, 1985; BODOY *et al.,* 1986). However, this index must be correlated with histological studies. To calculate this index, two techniques can be used to obtain the dry weight: oven drying (WALNE, 1976; MANN & GLOMB, 1978) and lyophilization (WALNE & MANN, 1975). BENINGER & LUCAS (1984), BODOY *et al.* (1986) and SAHIN *et al.* (2006) demonstrated similar results with these two methods for different bivalve species. Considering that the drying of tissues in the oven is easier to perform and more widespread due to standardization, this method was chosen. The condition index has proved to be effective for several species of bivalve molluscs with "severable" gonads, such as the oyster *Crassostrea gigas* (ENRIQUEZ-DIAZ, 2004) and the mussels *Mytilus edulis* (BRESSAN & MARIN, 1985) and *M. galloprovincialis* (YILDIZ *et al.,* 2006), as well as in species with "non-severable" gonads, such as the clams *Tapes decussates* and *T. phillipinarum* (LARUELLE, 1999) and *Crassostrea edule* (GUILLOU *et al.,* 1992). However, in some species, such as *Mesoderma mactroides* (HERMANN *et al.,* 2009), that have diffuse gonads, the condition index does not present a correlation with the gametogenetic cycle.

Case of the Bay of Tunis

The Bay of Tunis was our reference sampling site, with extended histological monitoring of reproduction over time. These data allowed us to evaluate the effectiveness of the condition index in *F. fragilis*. CHOI & CHANG (2009) reported that the condition index is not sufficient to determine the spawning season and that it must be accompanied by a histological study. In the Bay of Tunis, all indices of condition (CI_{Tot}, CI_{VM} and CI_R) show the same seasonal cycle. However, we note that the values for CI_{VM} were always greater than those for CI_R. The *F. fragilis* gonad is

diffuse and does not constitute a separate organ. However, variations in the CI_{VM} are mainly due to changes relating to increasing gonad volume during periods of gametogenetic activity. Generally, an increase in CI is an indicator of reserve accumulation during the period of sexual rest and gonadic development, whereas its reduction reflects gamete emission. This phenomenon was observed in several bivalve mollusk species: *Anadra granosa* (BROOM, 1983), *Spisula solida* (JOAQUIM *et al.,* 2008), *Donax trunculus* (GASPAR *et al.,* 1999) and *Cerastoderma edule* (GUILLOU *et al.,* 1992). However, although the CI was correlated with changes in the gametogenetic cycle in *F. fragilis*, the trends are reversed in this species, with the peak values in the index coinciding with spawning periods, mainly partial, and the falls coincided with sexual inactivity periods. This phenomenon was also observed for CI_{VM} and CI_R. We assume that there is a bioenergy competition between the gonadic and germinal compartments in this bivalve, a phenomenon already demonstrated in suspension feeding bivalves such as *Pecten maximus* (LUBET & MANN, 1987). In fact, the highest correlation observed between CI_R and gametogenetic stages was observed in the maturation stage ($r = 0.67$, $P = 0.004$, Pearson Product moment) for the confidence interval of 95%. This reflected a simultaneous increase in the somatic and germinal compartments. Thus, when conditions are favourable, mainly in terms of temperature and nutrient availability, this invasive cockle has a gamete maturation accompanied by an increase in non-visceral tissues.

Histological observations of the gonads showed an asynchrony in the gametogenetic stages of *F. fragilis*. These characteristics may explain the strong correlation between the CI_{VM} and partial spawning. The gonadic weight losses were negligible, since CI_{VM} increased during this stage and this reflects a low gamete emission over an elongated spawning period. This phenomenon has already been observed in *Donax trunculus* by GASPAR *et al*. (1999) on the Portuguese coast.

The CI also reflects metabolic balance changes caused by environmental factors. Previous studies have shown a correlation between this index and phytoplankton biomass, as in the cases of *Anadara inaequivalis* (SAHIN *et al.,* 2006) and *Laternula elliptica* (KANG *et al.,* 2009), or with sea temperature, as in the case of *Crassostrea edule* (GUILLOU *et al.,* 1992). For *F. fragilis,* the condition indices, under favourable reproduction conditions, are strongly correlated with the availability of nutrients and positively correlated with temperature. Indeed, we recorded seasonal peaks for the condition indices CI_{VM} and CI_R during periods of phytoplankton peaks in autumn (October and November 2006), summer (June 2006 and June 2007) and spring (May 2006 and April 2006) and a decrease in winter, with a minimum value in January, the month corresponding to the lowest concentration of chlorophyll-a (0.28 mg/L). In March 2007, following increases in temperature and chlorophyll-a, we noted a significant increase in CI, resulting in a significant percentage of spawning, which reflects the rapid transformation of the food ingested into gametes. This phenomenon was already described by THOMPSON & BAYNE (1972) in mussels and KANG *et al*. (2009) in the Atlantic clam *Laternula elliptica*. THOMPSON & BAYNE (1972) showed that the transfer of the energy contained the ingested food from the digestive gland to the gonad of the mussel took only seven days. In autumn 2007, all condition

indices showed a highly significant decrease, which was probably due to the high temperatures, close to sublethal (30°C). This phenomenon is therefore not related to the availability of food in the sea, because during this period the chlorophyll-a concentrations were relatively high.

Case of Bizerte Lagoon

The different condition indices of the Bizerte Lagoon population, particularly the CI_{VM}, reflected the gonadic development. The low values recorded during the period, which coincided with low gonadic activity (as determined by the histological study), can partly be explained by the pollution of the site. Declines in CI_R might therefore reflect physiological weakening due to pollution. McDOWELL *et al*. (1999) assume that the reductions of the "Scope for growth" in *Mytilus edulis* could be linked to the accumulation of aromatic hydrocarbons, inhibiting tissue growth during gonadic development. These authors also observed reduced fertilization, and abnormal gametic and embryonic development. Analysis of the different condition indices shows monthly changes of *F. fragilis* in Bizerte and demonstrates a metabolic imbalance caused by environmental stress. The high mortality rate of this Cardiidae confirmed this phenomenon.

In the literature, several studies have used the CI as an eco-physiological index reflecting the animal's condition and development (growth and reproduction) in a given environment. ROBELO *et al*. (2005) demonstrated that in the oyster *Crassostrea rhizophorae* this index was negatively correlated with the concentration of cadmium in the sediment. The same correlation was demonstrated in *Tapes decussatus* in the Gulf of Gabès (southern Tunisia) (SMAOUI-DAMAK *et al*., 2006). Similarly, MERZOUKI *et al*. (2009) demonstrated that the CI of *Mytillus galloprovincialis* was affected by heavy metal concentrations (i.e. zinc, copper, cadmium and mercury).

In Bizerte Lagoon, several studies of the affects of pollution on marine species have been published. We cite the case of three fish species (*Gobius niger, Gobius paganellus* and *Zosterisessor ophiocephalus*), which show skeletal deformities caused by high pollution of the lagoon, reflected in a deterioration of the metabolism of these animals (LOUIZ *et al*., 2007). In addition, LOUIZ *et al*. (2009) conducted a study on the relationship between gonad index and gonad histopathology of *G. niger,* a common species in Bizerte Lagoon. They demonstrated that low values of this index were correlated with gonadic abnormalities and negatively correlated with the level of pollution of the lagoon.

Comparing the environmental parameters at both sites, we found a significant difference between their dissolved oxygen concentrations. Monthly concentrations recorded at Bizerte Lagoon were statistically lower than those recorded in the Bay of Tunis (ANOVA, $p > 0.05$). WIDDOWS *et al*. (1997) and BAKER & MANN (1992) have shown that under conditions of anoxia or hypoxia, the total metabolism is reduced and higher levels of mortality are observed in mussels and oysters. In addition, several studies in invertebrate immunology have shown anoxia and hypoxia to be

causes of impaired immune systems, thus increasing liability to disease (BAKER & MANN, 1992; HARRIS *et al*., 1999; CHENG *et al*., 2004). Moreover, according to MAHMOUD *et al*. (2010), high concentrations of *Vibrionacea* were detected in the months of April and June 2006 in *F. fragilis* in Bizerte Lagoon. It should be noted that this family of bacteria includes several species of fresh and marine waters, some of which live in symbiosis with their hosts while others, are pathogenic. Adverse effects of this family of bacteria include nutritional stress inducing a chemotactic phenomenon that is the cause of fasting in the host species (SOTO *et al*., 2010). These data may also explain in part the variations in the condition index for *F. fragilis* in Bizerte Lagoon.

In future surveys, we plan to undertake in-depth histopathological studies and assays of contaminants in order to explain the ecophysiological differences in *F. fragilis* at these two study sites.

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