Journal MVZ Cordoba
2022; september-december. 27(3):e2695.
https://doi.org/10.21897/rmvz.2695

Original

Detection of *Perkinsus* sp. in *Chionista fluctifraga* cultivated in the southeast Gulf of California

Andrés M. Góngora-Gómez[1]; María F. Navarro-Chávez[1]; Lizeth C. Villanueva-Fonseca[2]; Brenda P. Villanueva-Fonseca[2]; Juan A. Hernández-Sepúlveda[1]; Ana L. Domínguez-Orozco1; Manuel García-Ulloa[1]*.

[1]Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Sinaloa, Guasave, Sinaloa, México.
[2]Universidad Autónoma de Occidente, Unidad Guasave, Guasave, Sinaloa, México.
*Corresponcia: turbotuag@hotmail.com

Received: March 2022; Accepted: August 2022; Published: September 2022.

ABSTRACT

Objective. To determine the presence of *Perkinsus* sp. in the sand clam *Chionista fluctifraga* cultivated in an intertidal zone of the southeastern Gulf of California, using Ray's thioglycollate fluid medium staining (MFTR) and polymerase chain reaction (PCR). **Material and Methods.** The prevalence and parasite load of *Perkinsus* sp. were monthly obtained and correlated with environmental water conditions and clam biometric indicators (n=540), from May 2018 to September 2019. **Results.** Presumptive hypnospores of the protozoan were detected in eight months of culture. The prevalence fluctuated from 3.3% to 13.3%, while the average parasite load ranged from 2 to 1286 hypnospores/g of tissue. The intensity of infection varied from negative to light. The prevalence and parasite load were correlated with each other ($r=0.61$, $p<0.05$) but not with the environmental parameters, nor with the biometric indicators of the clam. The PCR test was negative for the MFTR positive cases. **Conclusions.** Presumptive hypnospores of *Perkinsus* sp. were detected in *C. fluctifraga* without clear indications of infection that compromised the clam health in culture. The constant monitoring of *Perkinsus* sp. in *C. fluctifraga* is highly recommended to establish possible infections.

Keywords: Aquaculture; clam; Gulf of California; Mexico; parasitology; prevalence (Source*: MeSH, USDA*).

RESUMEN

Objetivo. Determinar la presencia de *Perkinsus* sp. en la almeja *Chionista fluctifraga* cultivada en una zona intermareal del sureste del Golfo de California, utilizando la tinción del medio fluido de tioglicolato de Ray (MFTR) y la reacción en cadena de la polimerasa (PCR). **Material y métodos.** La prevalencia y carga parasitaria de *Perkinsus* sp. se obtuvieron y se correlacionaron mensualmente con las condiciones ambientales del agua y los indicadores biométricos de la almeja (n=540), de mayo de 2018 a septiembre de 2019. **Resultados.** Se detectaron presuntas hipnosporas del protozoo en

How to cite (Vancouver).
Góngora-Gómez AM, Navarro-Chávez MF, Villanueva-Fonseca LC, Villanueva-Fonseca BP, Hernández-Sepúlveda JÁ, Domínguez-Orozco AL, García-Ulloa M. Detection of Perkinsus sp. in Chionista fuctifraga cultivated in the southeast Gulf of California. Rev MVZ Cordoba. 2022; 27(3):e2695. https://doi.org/10.21897/rmvz.2695

ISSNL: 0122-0268

ocho meses de cultivo. La prevalencia fluctuó de 3.3% a 13.3%; la carga parasitaria osciló entre 2 y 1286 hipnosporas/g de tejido. La intensidad de la infección varió de negativa a leve. La prevalencia y la carga parasitaria se correlacionaron positivamente entre sí (r=0.61, p<0.05), pero no con los parámetros ambientales, ni con los indicadores biométricos de la almeja. La prueba de PCR fue negativa para los casos positivos de MFTR. **Conclusiones.** Se detectaron presuntas hipnosporas de *Perkinsus* sp en *C. fluctifraga* sin claros indicios de infección que comprometieran la salud de la almeja en cultivo. Se recomienda el monitoreo constante de *Perkinsus* sp. en *C. fluctifraga*, para establecer posibles infecciones.

Palabras clave: Acuacultura; almeja; Golfo de California; México; parasitología; prevalencia (*Fuente: MeSH, USDA*).

INTRODUCTION

The mollusk industry represents an important source of food worldwide. According to the Food and Agriculture Organization of the United Nations (FAO), its cultivation contributes approximately 3.5 per cent (17.1 million tons) of its total annual production (1). In Mexico, several clam species are valued both for their demand for human consumption and for their aquaculture potential (2,3). However, one of the main "bottlenecks" in the development of their crop is disease.

Perkinsosis is a disease that has affected the world's bivalve mollusk industry (4), which is caused by different species of the *Perkinsus* protozoan and has sometimes been associated with massive mortalities (5). Such is the negative effect it causes on the cultivation of mollusks that this pathogen is considered by the World Organization for Animal Health as a parasite subject to compulsory declaration (6).In Mexico, *Perkinsus marinus* was reported infecting the Eastern oyster *Crassostrea virginica* in the Gulf of Mexico (7) and, for about two decades, the Cortez oyster *Crassostrea corteziensis* in the Mexican Pacific (8). For a few years, protozoa of the genus *Perkinsus* have been detected in various species of wild bivalve populations within the Gulf of California, such as the atrina *maura* (9) and the chocolate clam *Megapitaria squalide* (10). In cultures, the presence of *P. marinus* was confirmed in the smooth clam *Chionista fluctifraga* along with the Japanese oyster *Crassostrea gigas* on a farm in the northeast Gulf of California (11), confirming the horizontal dispersal of the pathogen between species in the area.

Recently, *C. fluctifraga* was polycultured with *C. gigas* and *M. squalida*,in an intertidal zone on the southeastern coast of the Gulf of California,with promising harvest time results (12). However, the health status of its culture in relation to the presence of this parasite is unknown, which would pose a risk to its production and other species (3). The purpose of this study is to evaluate the presence of *Perkinsus* sp. in smooth clam grown in El Colorado Bay, Ahome, Sinaloa, by detection and confirmation with the standard Ray Thioglycolate Fluid Medium (MFTR) technique and polymerase chain reaction (PCR). the indicators (prevalence, parasitic load, and intensity of infection) and their relationship with environmental conditions and clam biometrics are determined respectively.

MATERIALS AND METHODS

Collecting organisms and recording water parameters. Monthly (April 2018-September 2019), 30 clams of *Chionista fluctifraga* were collected from the intertidal culture plot belonging to the company SEAFARMERS S.A de C.V, located in El Colorado Bay, municipality of Ahome, Sinaloa. The plot is delimited by the following coordinates: 25° 43' 46.30"-25° 43' 38.70" N and 109° 33' 54.50"-109° 23' 47.80" W.

The specimens were transported alive in a container with sea water and aeration for one hour, to the Malacology Laboratory of Instituto Politecnico Nacional - Centro Interdisciplinario de Investigacion para el Desarrollo, Integral Regional (IPN-CIIDIR), under the standard rules (NOM-031-SSA1-1993, Goods and Services. Fishery products. Fresh- cooled and frozen bivalve mollusks). It should be noted that the Ethics Committee of the IPN-CIIDIR Teacher's Association authorized this research.

Also, in each sample, the temperature and dissolved oxygen of the water were obtained with an oximeter (YSI,5/12FT), pH with

a potentiometer (HANNA), salinity with a precision refractometer (ATAGO), and depth and transparency with a Secchi disk. Totalsuspended solids (SST), particulate organic matter (POM) and chlorophyll A concentration (Cl-*a*) were determined according to the gravimetric (13) and spectrophotometric (14) method for sea water.

Biometric and infectious indicators. For each clam, the length (maximum distance between the anterior and posterior margins), height (maximum distance from the umbo to the ventral margin), andthe width of the shell (maximum distance between the thickest parts of the two shells) were obtained with a digital vernier ruler (0.01 mm), plus total weight and soft tissue weight (0.01 g, wet weight) with a portable balance. Soft tissue was processed according to the standardRay Thioglycolate Fluid Medium (MFTR) technique to detect suspected*Perkinsus* sp. hypnospores. (6). Prevalence (number of clams with suspected hypnospore/total number of clams) X 100 (15), parasitic load = number of hypnospore per gram of clam tissue (16), and intensity of infection categorized as: Negative (0 hypnospores), mild ($<10^4$ hypnospores per gram of tissue), moderate (10^4 to 10^5 hypnospores per gram of tissue), and severe ($>10^5$ hypnospores per gram of tissue) (17).

For digestion, 4 g of clam tissue in tubes with MFTR was used to be incubated in darkness for 7 days at room temperature. When the tissue weighed less than 4 g (small clams), the whole body was used in digestion. Subsequently, 35 mL NaOH 2M was added to digest for 60 min at 60°C. Finally, the tubes were centrifuged (2.000 rpm per 3 min), NaOH 2M was discarded and rinsed with distilled water conserving the sediment of the sample (6). For DNA extraction from spores, samplesare washed with distilled water and, under a microscope (10X), the spore wall is broken with a fine-pointed needle. Finally, 1 mL of DNAzol® and 5 µL of proteinase K (6) are added to the sample (approximately 100 µL) .

DNA obtained from digestions and extractions of both, hypnospores and gill tissue, was used as white DNA in the electrophoretic PCR reaction (6). Oligonucleotides PerkITS85 (5'-CCG-CTT-TGT-TTG-GAT- CCC-3') and PerkITS750 (5'-ACA-TCA-GGC-CTT-CTA-ATG-3') were used to detect any species of *Perkinsus* sp. (18), and the specific oligonucleotides PmarITS-70F (5'-CTT-TTG-YTW-GAG-WGT-TGC-GAG- ATG-3') and PmarITS600R (5'-CGA-GTT-TGC-GAG-TAC-CTC-KAG-3') for *P. marinus* (19).

Genomic DNA from *C. corteziensis* infected with *P. marinus* (20) was used as a positive control, and water was added instead of DNA as a negative control.

Statistical analysis. Data that did not show a normal distribution (environmental parameters and infectious indicators, Kolmogorov-Smirnov) were transformed (square root) before statistically analyzing them. Multivariate correlations (*r*, Pearson) were applied between the infectious indicators and the rest of the parameters (environmental sampling site and biometrics corresponding to the clam) with a significance level of 0.05. The Statgraphics Centurion XVII program was used.

RESULTS

The ranges of water parameters were: Temperature = 15.9°C in February 2019 to 32.1°C in September 2019; salinity = 25 g salt/L inSeptember 2018 to 40 g salt/L in March 2019; dissolved oxygen = 5.14 mg/L in August 2018 to 9.63 mg/L in April 2018; PH = 4.3 in August 2018 to 8.21 in March 2019; depth = 0.20 m in March 2019 to 1.15 m in September 2019; transparency = 0.16 m in April 2019 to 0.8 m in October 2018 and September 2019; CL-*A* = 2.2 mg/m^3 in August 2019to 10.5 mg/m^3 in November 2018; SST = 19.3 mg/L in July 2018 to 189.2 mg/L in November 2018; and MOP = 4.8 mg/L in June 2018 to 26.3 mg/L June 2019. Table 1 shows biometric values of the clam for each month.

During light microscope analysis (x10 and x40) of clam tissue samples,dark blue round spherical corpuscles ranging from 20 to 70 µm in diameter (Figure 1) were detected with MFTR staining, standardcharacteristics of suspected protozoan hypnospores (4,6).

Prevalence and parasitic load had an interval of 0% to 13.33% and 2 to 1.286 hypnospores per gram of tissue (Figure 2). The intensity of infection in all months of cultivation was zero (April, July, September,October, November and December 2018, and January, February,March, May, June, July, august, and September 2019) to light (may and August 2018, and April 2019).

Table 1. Biometrics of *Chionista fluctifraga* (mean ± standard deviation) grown in the intertidal zone of El Colorado Bay, Ahome, Sinaloa, Mexico (April 2018-September 2019).

2018/ 2019	Length (mm)	Height (mm)	Width (mm)	Weight (g)
April	7.7 ± 0.9	7.2 ± 0.8	3.7 ± 0.4	0.10 ± 0.1
May	11.9 ± 1.2	10.5 ± 1.2	6.0 ± 0.7	0.50 ± 0.2
June	15.9 ± 1.2	13.9 ± 1.5	8.3 ± 0.8	1.26 ± 0.3
July	31.2 ± 1.5	26.7 ± 1.3	17.0 ± 0.9	9.33 ± 1.4
August	34.8 ± 1.8	30.1 ± 1.8	19.4 ± 1.1	13.16 ± 2.0
September	33.9 ± 2.2	28.7 ± 1.9	18.6 ± 1.2	11.84 ± 2.2
October	36.8 ± 1.6	31.7 ± 2.0	20.1 ± 1.0	14.69 ± 2.0
November	37.1 ± 2.2	32.0 ± 2.2	21.5 ± 3.1	15.76 ± 2.7
December	38.3 ± 2.5	32.9 ± 1.5	21.6 ± 1.1	17.58 ± 2.9
January	39.0 ± 2.0	32.9 ± 1.7	21.5 ± 1.3	18.62 ± 2.7
February	40.1 ± 1.6	34.6 ± 1.5	22.6 ± 1.8	20.34 ± 2.7
March	40.8 ± 2.4	35.2 ± 1.9	22.9 ± 1.9	21.75 ± 3.5
April	42.7 ± 1.8	36.4 ± 2.0	23.7 ± 1.7	23.30 ± 2.9
May	42.6 ± 2.7	36.7 ± 2.3	23.5 ± 1.9	23.18 ± 4.0
June	44.7 ± 5.9	37.9 ± 1.3	24.7 ± 1.1	26.65 ± 2.8
July	44.8 ± 2.3	38.0 ± 1.8	25.1 ± 1.4	27.12 ± 5.2
August	45.8 ± 3.0	38.5 ± 2.8	25.1 ± 1.7	28.78 ± 5.1
September	45.7 ± 2.7	38.4 ± 2.0	25.3 ± 1.5	28.63 ± 4.4

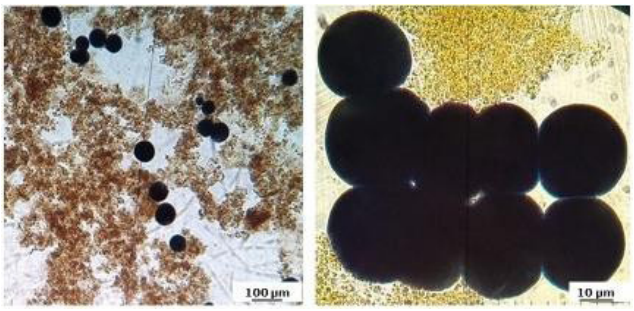

Figure 1. Observation of suspected hypnospores of *Perkinsus sp.* In the tissue of *Chionista fluctifraga*. Left: Light microscope (10X), bar scale = 100 µm; right: Light microscope (40x), bar scale = 10 µm.

Of the 19 clams that were positive for MFTR staining (3.5%), no confirmation was obtained for *Perkinsus* sp. or *P. marinus* with the PCRtest. There was no correlation between the infectious indicators with the water and biometrics parameters of the clam, but between the prevalence and parasitic load (r = 0.61, p<0.05).

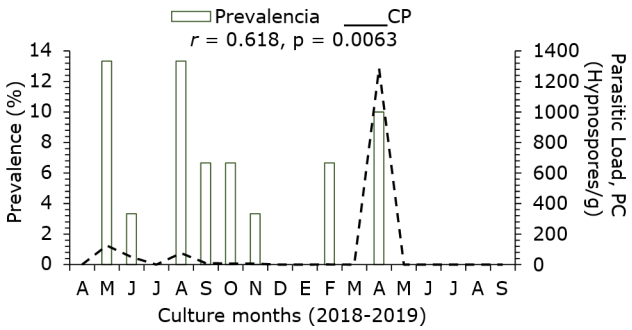

Figure 2. Prevalence (%) and parasitic load (hypnospores/g) of *Perkinsus* sp. In *Chionista fluctifraga* cultivated in the intertidal zone of El Colorado Bay, Ahome, Sinaloa, Mexico (April 2018-September 2019).

DISCUSSION

Although the recorded environmental parameters were within the range for clam cultivation (21), our values recorded a wider range to work reported in the same bay (22,23). This can be explained, in part, to the duration of the experimental times between investigations and to the inherent conditions of an intertidal zone culture, which suffers cyclical exposure to the weather. On the other hand, biometrics showed a constant growth of *C. fluctifraga* during the 18 months (12), with nosigns of involvement due to the environmental indicators recorded.

The different soft tissue components of *C. fluctifraga* showed no apparent characteristic Perkinsosis damage (watery and thin tissue, slow closing of leaflets and whitish corpuscles, 6) under visual and microscope observation. However, MFTR staining detected suspected protozoan hypnospores in clam tissue 8 months after culture, without a trend dictated by environmental parameters. This is consistent with the reports of other authors (20,27) studying various bivalves near thestudy area, who mention that infectious indicators were not correlated with fluctuations in water parameters, including temperature and salinity, the main indicators recognized as promoters of infection (4). The detection of hypnospores of *Perkinsus* sp. and *Perkinsus marinus* has previously been reported in different bivalve species in the Gulf ofCalifornia in Mexico (9,10), including the smooth clam (11), but its presence has not been associated with serious infections or mortalities.The average prevalence of *Perkinsus* sp. in *C. fluctifraga* obtained withMFTR (3.5%) was lower than that reported in a crop located north of the east coast

of the Gulf of California for the same species (11), As for other bivalve mollusk species in the Gulf of California (8,24). In addition, the average parasitic load of *Perkinsus* sp. in 18 months of smooth clam culture (195 hypnospores/g), associated with a slight intensity of infection, suggests that: 1) the environmental conditions of the site have favorable health characteristics for its cultivation (25),which is consistent with its classification as a certified area for the cultivation of mollusks, and, 2) in agreement with other clams (10,26) studied near the study area, *C. fluctifraga* shows no tissue damage from this pathogen (4).

The prevalence and parasitic load of the protozoan were not correlated with any environmental or biometric host parameter, which is consistent with that reported for *C. gigas* grown in the same bay (27). It is therefore possible to infer that the conditions of the site have remained stable, without affecting the health condition of the bivalves studied in that site. However, the results of this study are different from those reported for wild species of *M. squalida* south of the culturesite, where the protozoan infectious indicators did show correlation with the size of the chocolate clam and salinity (10,26). The difference in results with wild adult organisms could be explained by the longer period of time they have been exposed to the parasite before being sampled, in addition to the environmental conditions of each zone.

Despite the sensitivity and specificity of the PCR technique (6), the pathogen was not detected, probably due to the low values of the infectious indicators expressed in little or no genetic material (DNA) available for testing. this coincides with the degree of infection intensity of the clams, which was from zero to light. Staining with thioglycolate showed suspected parasite hypnospores, but with low prevalence andnumber of suspected hypnospores per gram of tissue. The difference found in both MFTR and PCR can be attributed to the amount of tissue tested in each test. For the thioglycolate staining test, 4 g of tissue wasused, whereas for the PCR test, approximately 50-100 mg was used inDNA extraction. The same was observed for other bivalve species in the region (9,27,28).

Since *C. fluctifraga* grew steadily, with no signs of infection, it is possible to conclude that the health status of its crop in the intertidal zone of the southeastern Gulf of California would not be compromised by the presence of *Perkinsus* sp. However, due to its dispersal and detection in different bivalves of the area, constant monitoring of the parasite in future crops and their surrounding areas is recommended.

Conflict of interest

The authors declare that there was no conflict of interests of any kind during the realization and elaboration of this work.

Acknowledgements

The authors are grateful for the logistical and financial support of Instituto Politecnico Nacional (IPN), the Secretariat of Research and Graduate Studies (SIP-IPN), the Commission for Operations and Promotion of Academic Activities (COFFA-IPN) and the Incentive for the Performance of Researchers (EDI-IPN), to carry out this study with the projects: SIP-IPN 20200526 and SIP-IPN 20210085.

REFERENCES

1. Naylor RL, Hardy RW, Buschmann AH, Bush SR, Cao L, et al. A 20-year retrospective review of global aquaculture. Nature. 2021; 591:551-563. https://doi.org/10.1038/s41586-021-03308-06

2. Flores-Garza R, García-Ibáñez S, Flores-Rodríguez P, Torreblanca-Ramírez C, Galeana-Rebolledo L, et al. Commercially important marine mollusks for human consumption in Acapulco, Mexico. Nat Res. 2012; 3:11-17. https://doi,org/10.4236/nr.2012.31003

3. Jiménez-Quiroz MC, Barrón-Barraza FJ, Cervantes-Duarte R, Funes-Rodríguez R. Environmental considerations for the management of the bivalve fisheries of Bahía Magdalena (Mexico), a coastal lagoon at the Southern end of the California current. Front Mar Sci. 2021; 8:682148. https://doi.org/10.3389/fmars.2021.682148

4. Dang C, de Montaudouin X, Binias C, Salvo F, Caill-Milly N, et al. Correlation between perkinsosis and growth in clams Ruditapes spp. Dis Aquat Org. 2013; 106:255-265. https://doi.org/10.3354/dao02640

5. Powell EN. What Is Going on with Perkinsus marinus in the Gulf of Mexico? Estuaries and Coasts. 2017; 40(1):105–120. https://doi.org/10.1007/s12237-016-0128-7

6. OIE. Manual de las Pruebas de Diagnóstico para los Animales Acuáticos. Capítulo 2.5.6 Infección por Perkinsus marinus. Organización Mundial de Sanidad Animal; 2019. http://www.oie.int/fileadmin/Home/esp/Health_standards/tahm/2.01.04_BOVINE_BRUCELL.pdf

7. Huicab-Pech ZG, Curiel-Ramírez S, Castañeda-Chávez M, Lango-Reynoso F, Carrillo-Alejandro P. Seasonal variation of Perkinsus marinus in the american oyster Crassostrea virginica from the carmen-machonapajonal lagoon system in tabasco, Mexico. Trop Subtrop Agroecosys. 2012; 15(2):S40–S50. https://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/1743

8. Cáceres-Martínez J, Vásquez-Yeomans R, Padilla-Lardizábal G, del Río Portilla MA. Perkinsus marinus in pleasure oyster Crassostrea corteziensis from Nayarit, Pacific coast of México. J Invert Pathol. 2008; 99(1):66–73. https://doi.org/10.1016/j.jip.2008.03.005

9. Góngora-Gómez AM, Rubio-Zepeda F, Villanueva-Fonseca LC, Álvarez-Dagnino E, Muñoz-Sevilla NP, et al. First record of Perkinsus sp. (Protozoa, Apicomplexa) in the pen shell Atrina maura in Sinaloa, Mexico. Rev Biol Mar Ocean. 2016; 51(3):689–694. https://doi.org/10.4067/S0718-19572016000300020

10. Góngora-Gómez A, Villanueva-Fonseca L, Sandoval-Rivera P, Hernández-Sepúlveda J, Domínguez-Orozco A, et al. Reporte de presuntas hipnosporas de Perkinsus sp. en Megapitaria squalida del Golfo de California con la técnica de tinción con tioglicolato. Rev MVZ Córdoba. 2020; 25(2):e1805. https://doi.org/10.21897/rmvz.1805

11. Enríquez-Espinoza TL, Castro-Longoria R, Mendoza-Cano F. Grijalva-Chon JM. Perkinsus marinus in Crassostrea gigas and Chione fluctifraga from Kino Bay, Sonora, Mexico. Biotecnia. 2015; 17(1):10–13. http://biotecnia.unison.mx/index.php/biotecnia/article/view/6.

12. Góngora-Gómez AM, Acosta-Campos MJ, Navarro-Chávez MF, Rodríguez-González H, Villanueva-Fonseca LC, et al. Optimizing harvest time through absolute and relative growth of the black clam, Chionista fluctifraga, cultivated in the intertidal along the southeastern coast of the Gulf of California. Turkish J Fish Aqua Sci. 2022; 22(2):19537. http://doi.org/10.4194/TRJFAS19537

13. Rice EW, Baird RB, Eaton AD, Clesceri LS. Solids (2540). Standard methods for the examination of water and wastewater 2017. American Public Health Association: Washington; 2021. https://yabesh.ir/wp-content/uploads/2018/02/Standard-Methods-23rd-Perv.pdf

14. Davies CH, Ajani P, Armbrecht L, Atkins N, Baird ME, et al. Data Descriptor: A database of chlorophyll *a* in Australian waters. Sci DATA. 2018; 5:180018. https://doi.org/10.1038/sdata.2018.18

15. Dantas Farias N, de Oliveira NFP, da Silva PM. Perkinsus infection is associated with alterations in the level of global DNA methylation of gills and gastrointestinal tract of the oyster Crassostrea gasar. J Invert Pathol. 2017; 149:76-81. https://doi.org/10.1016/j.jip.2017.08.007

16. Fisher WS, Oliver LM. A whole-oyster procedure for diagnosis of Perkinsus marinus disease using Ray's fluid thioglycollate culture medium. J Shell Res. 1996; 15(1):109-118.

17. Bushek D, Ford SE, Allen SK. Evaluation of methods using ray's fluid thioglycollate medium for diagnosis of Perkinsus marinus infection in the eastern oyster, Crassostrea virginica. An Rev Fish Dis. 1994; 4:201–217. https://doi.org/10.1016/0959-8030(94)90029-9

18. Casas SM, Villalba A, Reece KS. Study of perkinsosis in the carpet shell clam Tapes decussatus in Galicia (NW Spain). I. Identification of the ethiological agent and in vitro modulation of zoosporulation by temperature and salinity. Dis Aqua Org. 2002; 50(1):51-65. https://doi.org/10.3354/dao050051

19. Audemard C, Reece KS, Burreson EM. Real-time PCR for detection and quantification of the protistan parasite Perkinsus marinus in environmental waters. Appl Environ Microbiol. 2004; 70(11):6611-6618. https://doi.org/10.1128/AEM.70.11.6611-6618.2004

20. Villanueva-Fonseca LC, García-Ulloa M, López-Meyer M, Villanueva-Fonseca BP, Hernández-Sepúlveda JA, Muñoz-Sevilla NP, Góngora-Gómez AM. Perkinsus marinus in the pleasure oyster Crassostrea coerteziensis cultivated on the southeast coast of the Gulf of California, Mexico. Lat Am J Aquat Res. 2020; 48:529-537. http://doi.org/10.3856/vol48-issue4-fulltext-2463

21. Montagnac V, Guyondet T, Comeau L, R Tremblay. Physiological differences between wild and cultured bivalves in Prince Edward Island, Canada. Aquat Living Resour. 2020; 33:8. https://doi.org/10.1051/alr/2020008

22. Villanueva-Fonseca BP, Góngora-Gómez AM, Muñoz-Sevilla NP, Domínguez-Orozco AL, Hernández-Sepúlveda JA, et al. Growth and economic performance of diploid and triploid Pacific oysters Crassostrea gigas cultivated in three lagoons of the Gulf of California. Lat Am J Aquat Res. 2017; 45:466-480. http://doi.org/10.3856/vol45-issue2-fulltext-21

23. Escamilla-Montes R, Diarte-Plata G, Ruiz-Verdugo CA, Luna-González A, Vázquez-López H, et al. Patrones de crecimiento y proporción de sexos de la jaiba Callinectes bellicosus (Decapoda: Portunidae). Hidrobiológica. 2018; 28(3):301-311. https://doi.org/10.24275/uam/izt/dcbs/hidro/2018v28n3/diarte

24. Cáceres-Martínez J, Ortega MG, Vásquez-Yeomans R, García TDJP, Stokes NA, Carnegie RB. Natural and cultured populations of the mangrove oyster Saccostrea palmula from Sinaloa, México, infected by Perkinsus marinus. J Invert Pathol. 2012; 110(3):321–325. https://doi.org/10.1016/j.jip.2012.03.019

25. Diarte-Plata G, Escamilla-Montes R, Ruiz-Verdugo CA, Luna-González A, De-La-Cruz-Agüero G, et al. The growth of Callinectes arcuatus (Ordway, 1863) in the El Colorado Lagoon, Ahome, Sinaloa, Mexico. Lat Am J Aquat Res. 2018; 46(5):1041-1054. http://dx.doi.org/10.3856/vol46-issue5-fulltext-17

26. Góngora-Gómez AM, Villanueva-Fonseca LC, Arzola-González JF, Sotelo-López F, Hernández-Sepúlveda JA, García-Ulloa M. Presuntas hipnosporas de Perkinsus sp. en la almeja Megapitaria squalida del golfo de California. AquaTechnica: Rev Iberoame Acui. 2019a; 1(1):47–52. https://doi.org/10.33936/at.v1i1.2152

27. Góngora-Gómez AM, Sandoval-Rivera PA, Villanueva-Fonseca LC, Lopez-Meyer M, Sainz-Hernández JC, et al. Prevalencia de Perkinsus sp. (Apicomplexa) en la almeja chocolata Megapitaria squalida (Sowerby, 1835) de la costa central de Sinaloa, México. Hidrobiológica. 2019; 29(3):129-136. https://doi.org/10.24275/uam/izt/dcbs/hidro/2020v29n3/Gongora

28. Villanueva-Fonseca LC, Escobedo-Bonilla CM. Prevalence of the protozoan Perkinsus sp. in cultured Pacific oyster Crassostrea gigas in Sinaloa, Mexico. Lat Ame J Aquat Res. 2013; 41(5):996–1002. https://doi.org/103856/vol41-issue5-fulltext-19