Uni-edit Sample of Level 3 Editing (Biology)

Comment [A1]:
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Material and Methods

Field survey

Manila clams (~~N~~n=30) were collected monthly ~~collected~~ from a tidal flat off Tokuyama, Yamaguchi Prefecture, western Seto Inland Sea (~~A in~~ Fig. 2, Location A) from June 2004 to May 2005.~~Collected clams were kept in running seawater for two or three days and then transported to our laboratory in refrigerator overnight. Few clams died during the storage and transportation. Clams were measured at the~~ Sshell length (SL ~~in~~; mm), ~~shell~~ height (SH ~~in~~; mm) ~~, shell width~~and width (SW ~~in~~; mm) was measured prior to removal of ~~. S~~soft tissue~~. was removed from clams and~~ The wet soft tissue weight (WST ~~in~~; mg) was then recorded ~~weighted~~ after removing excess fluid by placing them on filter papers and a~~the~~ ~~. c~~Condition index (CI) was calculated using the following formula: WST/(SL x SH x SW)x1000.

The intensity of *Perkinsus* infection was quantified by conventional Ray's fluid thioglycollate medium (FTM) ~~following~~ according to Choi et al. (1989) and Almeida et al. (1999). Briefly, the outmost left or right gill leaf was removed from each clam, weighed after removing the excess moisture on filter papers, and subsequently incubated in Ray's FTM medium at 25 ^{o}C for one week. Incubated gills were treated –in 2N NaOH at 60 ^{o}C until the gills were lysed ~~, mostly within 30 min,~~ and then washed three times in PBS with centrifugation (1600xg, 15 min).

Comment [A2]: CHECK: Please write the acronym in full, followed by (PBS), the first time it is used.

Resultant pellets were suspended in 1 mL of PBS and a total of 10 µL of the resuspension was ~~observed~~ used to calculate the infection load. ~~with an inverted microscope for counting prezoosoprongia.~~ Considering the weight of gill leaves, detection sensitivity ~~of the method~~ was estimated at approximately 10^3 cells/g gill tissue. ~~This quantification protocol was employed throughout the present study unless otherwise stated~~

After removing the outmost gill leaf for examination of infection intensity, the remaining soft tissue was transversally cut ~~in half~~ into anterior and posterior parts. The ~~cut surface of the~~ posterior part was impregnated on glass slides and the anterior part was fixed in 10% buffered formalin for histological examination. Impregnated tissue preparations were stained using a commercial cytostaining kit (Diff-Quick staining, Symex International Reagents Co. Ltd, Kobe, Japan). For histological examination, a tissue slice of approximately 5 mm thickness was excised out from each of the fixed tissue, embedded in paraffin, sectioned at 5 µm and stained using hematoxylin and eosin. Impregnation preparations and histological sections were observed ~~with a light microscope~~ for ~~examination of~~ sporocysts of trematodes and sexual development of clams, respectively. Sexual development was categorized into ~~4~~ four stages: undifferentiated, developing, spawning and spent stages.

Comment [A3]: CHECK: Why are there six mentioned on the graph?

Effects of Perkinsus infection on physiological conditions of clams

The clearance rate, borrowing activity and tolerance against high water temperature were examined together with infection intensity of clams purchased from a local clam farm in Ohno (~~B on~~ Fig. 2, Location B~~),~~). At this location, ~~where~~ the level of *Perkinsus* sp. infection ~~level~~ was ~~known~~ high and both *P. olseni* and *P. honshuensis* were ~~present~~ detected (Takahashi et al., submitted). ~~Clams were transported in refrigerator overnight to t~~ Clearance rates and tolerance against high

water temperature were examined at The Fisheries Laboratory, ~~the~~ University ~~on~~ of Tokyo, ~~located on an inlet Hamana Lake,~~ Shizuoka Prefecture (~~C in~~ Fig. 2, Location C), while borrowing activity was examined at ~~where clearance rates and tolerance against high water temperature were examined, while clam were also transported in refrigerator overnight to our laboratory~~ at the Hongo Campus, ~~the~~ University of Tokyo, ~~located in~~ Tokyo (~~D in~~ Fig. 2, Location D)~~, where the borrowing activity was examined.~~.

Clearance rates were measured in 30 clams (SL, 33.2-44.3 mm) with the indirect method in August. Before measurements, clams were acclimatized ~~to~~ in running seawater at 20^{o}C for 24 hr. For measurements, individual clams were ~~individually~~ placed in a ~~in seawater in a~~ chamber ~~of~~ containing 500 ml of seawater ~~,~~ and were continuously given commercially cultured ~~diatom,~~ *Chaetoceros calcitrans* ($6.3x10^4$-$2.9x10^5$ cells/ml) (Sunculture, Nisshin Marintech, Aichi) ~~continuously~~ through a peristaltic pomp. Diatom densities of the inflow (C_{in} cells/ml) and outflow (C_{out} cells/ml) ~~of the chambers~~ were measured with hemocyte counting chambers. The flow rate (Fr ml/min) of the peristaltic pomp was adjusted between 440-900 ml/h to keep C_{out} between 10^4 - 10^5 cells/ ml. Clearance rates (Cr) were calculated with the following formula: Cr (ml/min) = Fr x (C_{in}-C_{out})/C_{in}. As ~~the~~ clearance rates ~~was found to became stable~~ stabilized within 2 hr ~~after~~ of transferring ~~them~~clams ~~clams were transferred~~into ~~in~~ the chambers ~~in our preliminary experiment~~, ~~the measurement of~~ C_{out} and C_{in} ~~was carried~~ was measured ~~out~~ 5 times every ~~one~~ hour from 2 hr after transfer of clam into the chambers. After the experiment, infection intensity in the left outmost gill leaf was examined with Ray's FTM.

For ~~examination of the~~ tolerance against high temperature, clams collected from Lake Hamana, which were moderately infected with *Perkinsus* ~~at a medium level~~, were compared against ~~also used together with~~ heavily infected clams ~~transported~~

Comment [A4]: CHECK: This information is not required.

Comment [A5]: CHECK: How were they measured during the other months??

Comment [A6]: CHECK: Please try to make this sentenceclearer.

~~from the clam farm located on~~from the Seto Inland Sea ~~in August~~. Initial infection intensity and condition indexes were ~~examined~~ recorded for ~~in~~ 30 individual clams from each population using ~~s of clams in each group with~~ Ray's FTM. Seventy six clams ~~each~~ collected from Lake Hamana and Seto Inland Sea were then ~~from the two groups were~~ placed in separate baskets ~~placed together~~ within~~in~~ a 40 L tank, ~~in~~ through which temperature-controlled ~~flow through~~ seawater was ~~given~~passed. Water temperature was gradually ~~raised~~ increased from 22 ^{o}C to 28 ^{o}C ~~in~~ over a four day period ~~4 days,~~ and ~~subsequently~~ then maintained at 28 ^{o}C for 8 days. ~~. During the experiment,~~ Clam survival was recorded twice a day with ~~clams were observed twice a day and dead and~~ moribund or dead clams ~~were~~ being removed from the tank. After the experiment, infection intensity ~~in~~ of the outmost gill leaf was examined with Ray's FTM.

Borrowing activity was examined ~~in~~ for 30 clams ~~transported~~ from ~~the clam farm on~~ Seto Inland Sea in June. Before the examination, clams were acclimatized at 20^{o}C for one week and fed diatom suspension. ~~For examination, e~~Each clam was then ~~individually~~ placed in a 1 L seawater tank with quartzose sand set and placed on the bottom of 100 L recirculating tanks. Water temperature was maintained at 20 ^{o}C. Borrowing of clams was observed continuously for ~~initial~~ 2 hrs, then every 30 min for the next 4 h~~our~~s and once at 24 h~~our~~s ~~after the begging of the experiment~~. The time taken for ~~Timing when~~ each clam shell to ~~completely~~ disappear~~ed~~ under the sand was recorded. After the experiment, infection intensity in the outmost gill leaf was examined with Ray's FTM.

Statistical analyses

One-way ANOVA followed by Tu~~r~~key-Kramer HSD test was used for multiple comparisons. Student's t-test was used for comparison between two groups.

Comment [A7]: CHECK: Please specify exactly which comparisons were made using ANOVA. Also indicate if a test for homogeneity of variance was performed before the ANOVA.

Comment [A8]: CHECK: Please specify which two groups were compared using Student's t-test.

Intensity was log-transformed prior to those analyses. Kendall's rank test was used for detection of correlation. Chi-square test was used for analyses of mortalities. In all the analyses, 5% was used as a significant level.

Comment [A9]: CHECK: Please specify why the data needed to be transformed. For example, you could write "the intensity data was log-transformed in order to satisfy the assumption of homogeneity of variance".