# Oral Presentation

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# Water purification abilities of eight Japanese Unionoida

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**Abstract:** The water purification capacities of eight species of unionid mussels, *Margaritifera laevis*、*Unio douglasiae nipponensis*, *Inversiunio jokohamensis*, *Lanceolaria grayana*, *Obovalis omiensis*, *Inversidens brandti*, *Anodonta japonica* and *Cristaria plicata* were determined through laboratory investigation. Under experimental conditions, all eight species demonstrated higher nocturnal than diurnal filtration rates. Furthermore, all species had a tendency to decrease total organic carbon (TOC) and total nitrogen (TN), demonstrating their contribution to water purification. However, no direct correlation was observed for any species in the relationship between filtration rate and TOC and TN removal rates, suggesting interspecies differences in TOC and TN removal capabilities.

**Key words:** water purification, unionid mussel, total organic carbon, total nitrogen, turbidity

## Introduction

Great numbers of filter-feeding bivalves in ocean, brackish and freshwater areas contribute to improvement in water quality through consumption of aquatic suspended matter, including phytoplankton, which are a cause of reduced water transparency. Much research has been conducted on the water purification capacity of filter-feeding bivalves with particular progress being made in studies on commercially important species such as Asari, *Ruditapes philippinarum*, and Yamato-shijimi, *Corbicula japonica* (Aizaki and Fukuchi, 1998; Isono, 1998, and many others). However, in contrast to the numerous studies on several marine bivalves, research into freshwater bivalves, which are not widely used as aquatic resources, is limited (Kryger and Riisgard, 1998; Sylvester *et al.*, 2005; Wu *et al.*, 2005).

The topography of Japan is mountainous with no large rivers or lakes and short rivers; therefore, the variety of freshwater mussels is more restricted than that on the continents of China and North

America. Basic data regarding habitat distribution and ecological characteristics of unionid mussels has been lacking due to the small number of researchers of these particular bivalves; however, recent collection of data has been increasing (Kondo, 2008). Considering that unionid mussels, which belong to the larger class of filter-feeding freshwater bivalves, are anticipated to have equal or greater purification capability than the commercially important *R. philippinarum* and *C. Japonica*, clarification of their filtration capacities is important with regard to water quality management of rivers, lakes and marshes.

Most research into the water purification capacity of bivalves has relied on calculated filtration rates based on turbidity (Coughlan, 1969; Nakamura *et al.*, 1988; Aizaki *et al.*, 2001); few have analyzed the removal of bioelements that cause contamination. Therefore, in the present study, we investigated the water purification capacity of freshwater bivalves (unionid mussels), which have rarely been used as experimental models to date, through laboratory experiments focusing on removal of turbdity, chlorophyll *a* (Chl.a), carbon and nitrogen.

## Methods

Seven species of unionid mussel comprising *Unio douglasiae nipponensis*, *Inversiunio jokohamensis*, *Lanceolaria grayana*, *Obovalis omiensis*, *Inversidens brandti*, *Anodonta japonica* and *Cristaria plicata* were collected from rivers, lakes and marshes in the Tokai and Kinki regions of Japan and one species, *Margaritifera laevis*, was collected in Hokkaido. These eight species of bivalves were reared with a sufficient daily supply of the alga *Chlorella vulgaris* according to the methods of Kryger and Riisgard (1988) and Sylvester *et al.* (2005). As many bivalves are sensitive to environmental disturbances, it was important to minimize the effects of changes in water temperature and stress of collection (McLusky, 1973; Jorgensen, 1975; Yamamuro, 1992); therefore, they were gradually acclimated in aquaria in the laboratory for ≥30 days prior to the start of experiments.

Experiments were conducted in commercially available plastic containers 270 mm (length) x 180 mm (width) x 120 mm (depth) and 2.5 L of pure *C. vulgaris* culture was added to achieve a Chl.a concentration of approximately 400 μg $L^{-1}$. Cultures were maintained at 20 ± 1.5°C with aeration

and a moderate water current was created to maintain a homogenous and suspended distribution of *C. vulgaris* throughout the experiments. In experiments on marine bivalves, differences in purification capacity between light and dark conditions have been observed (Kawase *et al.*, 2008). Therefore, in the present study four containers per species were placed in either light, dark. In the light condition, two regular 40 W fluorescent lights (2000 Lx) were applied. In the dark condition, the container was covered in a double layer of aluminum foil to exclude light. Control containers were maintained in each of the light conditions without mussels. Under natural conditions, freshwater bivalves usually burrow into sediment; however, as addition of sediment would result in elution of substances from the sediment, we conducted the present experiments without sediment.

Experiments were conducted with three to five mussels of each species in each experimental container to account for the possibility of individual differences in purification capacity. Table 1 shows the number of mussels used; mean shell length (length from the anterior to posterior margin) and standard deviation; and, mean wet weight and standard deviation for each experiment.

**Table 1. Experimental conditions of eight unionid mussel species**

| Japanese common name | Scientific name | Light condition | No. of mussels used in each experiment | Shell length | Shell length | Wet weight | Wet weight |
|---|---|---|---|---|---|---|---|
| | | | | Mean (mm) | Standard deviation | Mean (g) | Standard deviation |
| Kawashinju-gai | *Margaritifera laevis* | Light | 5 | 62.2 | 2.39 | 3.2 | 0.91 |
| | | Dark | 5 | 60.4 | 3.21 | 2.9 | 0.63 |
| Ishi-gai | *Unio douglasiae nipponensis* | Light | 4 | 53.3 | 0.26 | 2.5 | 0.41 |
| | | Dark | 4 | 52.0 | 0.42 | 2.1 | 0.14 |
| Yokohamashijira-gai | *Inversiunio jokohamensis* | Light | 4 | 67.5 | 3.70 | 5.3 | 0.88 |
| | | Dark | 4 | 66.3 | 4.99 | 5.3 | 0.99 |
| Sasanoha-gai | *Lanceolaria grayana* | Light | 5 | 66.4 | 5.64 | 1.1 | 0.23 |
| | | Dark | 5 | 68.8 | 5.76 | 1.4 | 0.44 |
| Kataha-gai | *Obovalis omiensis* | Light | 4 | 72.5 | 4.12 | 6.0 | 1.38 |
| | | Dark | 4 | 69.3 | 4.86 | 7.7 | 2.79 |
| Obaeboshi-gai | *Inversidens brandti* | Light | 3 | 40.7 | 2.08 | 2.3 | 0.12 |
| | | Dark | 3 | 40.0 | 0.00 | 2.1 | 0.53 |
| Ta-gai | *Anodonta japonica* | Light | 4 | 77.8 | 4.65 | 11.4 | 0.98 |
| | | Dark | 4 | 74.8 | 4.65 | 8.5 | 1.95 |
| Karasu-gai | *Cristaria plicata* | Light | 1 | 152.0 | – | 53.38 | – |
| | | Dark | 1 | 144.0 | – | 53.36 | – |

The direct method of flow rate measurement involves attachment of a glass tube to the exhalant and inhalant siphons of the bivalves to directly measure the water flow rate (Coughlan and Ansell, 1964). However, unlike *T. japonica* and *C. japonica*, the unionid mussels used in the present study do not possess siphons, having instead a sealed mantle structure (Masuda and Uchiyama, 2004). As the direct method is therefore likely to result in measurement errors, an indirect method often used to

measure filtration rate in bivalves was adopted (Yamamuro, 1992). Water was sampled and measured hourly over 6 h for a total of seven times per experiment. During sampling, care was taken to avoid disruption of mucus-bound feces and pseudofeces.

Measurement parameters, equipment and methods are as follows. Turbidity was quantified based on the kaolin turbidity standard solution using a spectrometer equipped with an integrating sphere attachment (V-550; JASCO). Chl.a was measured by vacuum filtration of the water sample through a glass fiber filter (GF/F; Whatman) followed by acetone extraction and fluorometric detection (10-AU, TURNER) based on the Lorentzen method. For analysis of total organic carbon (TOC), dissolved organic carbon (DOC), total nitrogen (TN) and total dissolved nitrogen (TDN), 200 μL of HCl was added to the samples and the dry method (combustion at 850°C) was used with analysis on a TOC Analyzer (TOC-V, TNM-1; Shimadzu).Total carbon (TC) was calculated as TOC + inorganic carbon (IC). Adding HCl removes IC, giving TC = TOC. Accordingly, TOC = particulate organic carbon (POC) + DOC. Conversely, TN = particulate organic nitrogen (PON) + TDN and TDN = dissolved organic nitrogen (DON) + total inorganic nitrogen (T inorg. N). Accordingly, T inorg. N = $NH_4 + NO_2 + NO_3$.

Filtration rate, $F$, is calculated by substituting turbidity values into equation (1), as used by Nakamura *et al.* (1988).

$$F = \frac{V}{T}\left(\ln\frac{C_0}{C_t} - \ln\frac{C_{b0}}{C_{bt}}\right) \quad (1)$$

where, $F$ is filtration rate (ml $h^{-1}$); $V$ is volume of water used in the container (ml); $T$ is duration of experiment (h); $C_o$ is concentration of the substance being measured at the start of the experiment in the container housing the mussels (mg $L^{-1}$); $C_t$ is the concentration of the substance being measured at $t$ h after the start of the experiment in the container housing the mussels (mg $L^{-1}$); $C_{bo}$ is the concentration of the substance being measured at the start of the experiment in the control container (mg $L^{-1}$); and, $C_{bt}$ is the concentration of substance being measured at $t$ h after the start of the experiment in the control container (mg $L^{-1}$).

Moreover, if equation (1) is transformed as in equation (2) below to become nondimensional, substituting the measurement values into the left side of the equation shows the efficacy of removal and aggregation of substances inside the containers. In Figs. 1 to 4, the vertical axis represents the results converted to mean individual values.

$$\frac{C_t}{C_0} \Big/ \frac{C_{bt}}{C_{b0}} = \exp\left(-\frac{F}{V}T\right) \tag{2}$$

## Results

Figure 1 shows the efficacy of removal and aggregation of suspended matter as changes in turbidity over time. Figure 2 shows the efficacy of Chl.a removal and aggregation as changes in Chl.a over time. Figure 1 clearly shows that all eight species of mussels reduced the volume of suspended matter in both light and dark conditions. With regard to the rate of change in Chl.a, Fig. 2 shows a general decreasing trend in rate of reduction for all eight species in both the light and dark conditions. Figures 1 and 2 show that rate of suspended matter and Chl.a removal and aggregation differed depending on the species. For example, peak reduction was reached 4 h after the start of the experiment for *M. laevis* regarding Figure 1, and 3 h after the start of the experiment for *I. jokohamensis* regarding Figure 2, after which an increasing trend was observed. *A. japonica* reduced turbidity at a mostly stable rate, while *O. omiensis* reduced Chl.a up to 1 h after the start of the experiment after which an increase was observed followed by a sharp decrease. In order to observe average trends during the experiments, Table 2 shows filtration rates obtained by substituting changes in turbidity in experimental sample into Eq. 1. Comparison of the filtration rates of the eight mussel species both per individual and per unit of wet weight reveals that the filtration rate was greater for all species in the dark rather than the light condition. Furthermore, the relative magnitude relationship between individual filtration rate and that per unit wet weight is not constant, showing interspecific differences under different experimental conditions.

Table 2. Rates of filtration and TOC and TN removal of eight unionid mussel species

| Japanese common name | Light condition | Filtration rate | | TOC removal rate | | TN removal rate | |
|---|---|---|---|---|---|---|---|
| | | per individual (ml $ind^{-1}h^{-1}$) | per unit wet weight (ml $g^{-1}h^{-1}$) | per individual (ml $ind^{-1}h^{-1}$) | per unit wet weight (ml $g^{-1}h^{-1}$) | per individual (ml $ind^{-1}h^{-1}$) | per unit wet weight (ml $g^{-1}h^{-1}$) |
| Kawashinju-gai | Light | 135.1 | 42.3 | 0.63 | 0.197 | 0.16 | 0.051 |
| | Dark | 142.8 | 49.3 | 0.70 | 0.241 | 0.21 | 0.074 |
| Ishi-gai | Light | 127.3 | 51.5 | 0.48 | 0.192 | 0.08 | 0.032 |
| | Dark | 125.5 | 60.5 | 0.53 | 0.256 | 0.10 | 0.049 |
| Yokohamashijira-gai | Light | 104.9 | 19.9 | 1.21 | 0.229 | 0.20 | 0.037 |
| | Dark | 143.5 | 27.3 | 0.59 | 0.112 | 0.15 | 0.029 |
| Sasanoha-gai | Light | 54.8 | 49.5 | 0.14 | 0.130 | 0.03 | 0.023 |
| | Dark | 76.3 | 54.4 | 0.25 | 0.176 | 0.04 | 0.029 |
| Kataha-gai | Light | 105.4 | 17.4 | 0.92 | 0.151 | 0.07 | 0.012 |
| | Dark | 172.8 | 22.4 | 0.85 | 0.110 | 0.22 | 0.028 |
| Obaeboshi-gai | Light | 66.3 | 29.1 | 0.54 | 0.236 | 0.13 | 0.056 |
| | Dark | 82.0 | 38.7 | 1.01 | 0.477 | 0.28 | 0.132 |
| Ta-gai | Light | 107.7 | 9.4 | 0.88 | 0.077 | 0.18 | 0.016 |
| | Dark | 131.6 | 15.4 | 1.15 | 0.134 | 0.25 | 0.029 |
| Karasu-gai | Light | 252.7 | 4.7 | 1.09 | 0.020 | 0.40 | 0.007 |
| | Dark | 524.0 | 9.8 | 2.56 | 0.048 | 0.89 | 0.017 |

Efficacy of TOC, DOC and POC (POC = TOC – DOC) removal and aggregation is shown in Fig. 3 while that for TN, TDN and PON (PON = TN – TDN) is shown in Fig. 4. A small difference in TOC removal was seen between light and dark conditions but a decreasing trend was observed for all species. However, for many species, the decrease in TOC mainly comprised POC, with little change observed in DOC. TN showed similar overall trends as TOC, with slight differences between light and dark conditions but a decreasing trend in TN for all species. The decreasing TN was mainly PON; many species showed a slight increase in TDN. Table 2 shows hourly TOC and TN removal rates calculated based on Figs. 3 and 4. Comparison of the TOC removal rate per individual between the eight mussel species reveals that under light conditions, *I. jokohamensis* has the highest removal rate while under dark conditions, *C. plicata* and *A. japonica* have the highest values. *I. jokohamensis* and *O. omiensis* have higher TOC removal rates in light conditions than dark conditions while the other six species showed higher removal rates under dark conditions. With the exception of *I. jokohamensis*, all species showed higher TN removal rates under dark rather than light conditions. Comparison of the TN removal rate per individual for each species reveals that under both light and dark conditions, *C. plicata* had the highest values. However, as with filtration rate, there was no constant relative magnitude relationship between the TOC and TN removal rates per unit of wet weight and that per individual, with each species displaying different characteristics.

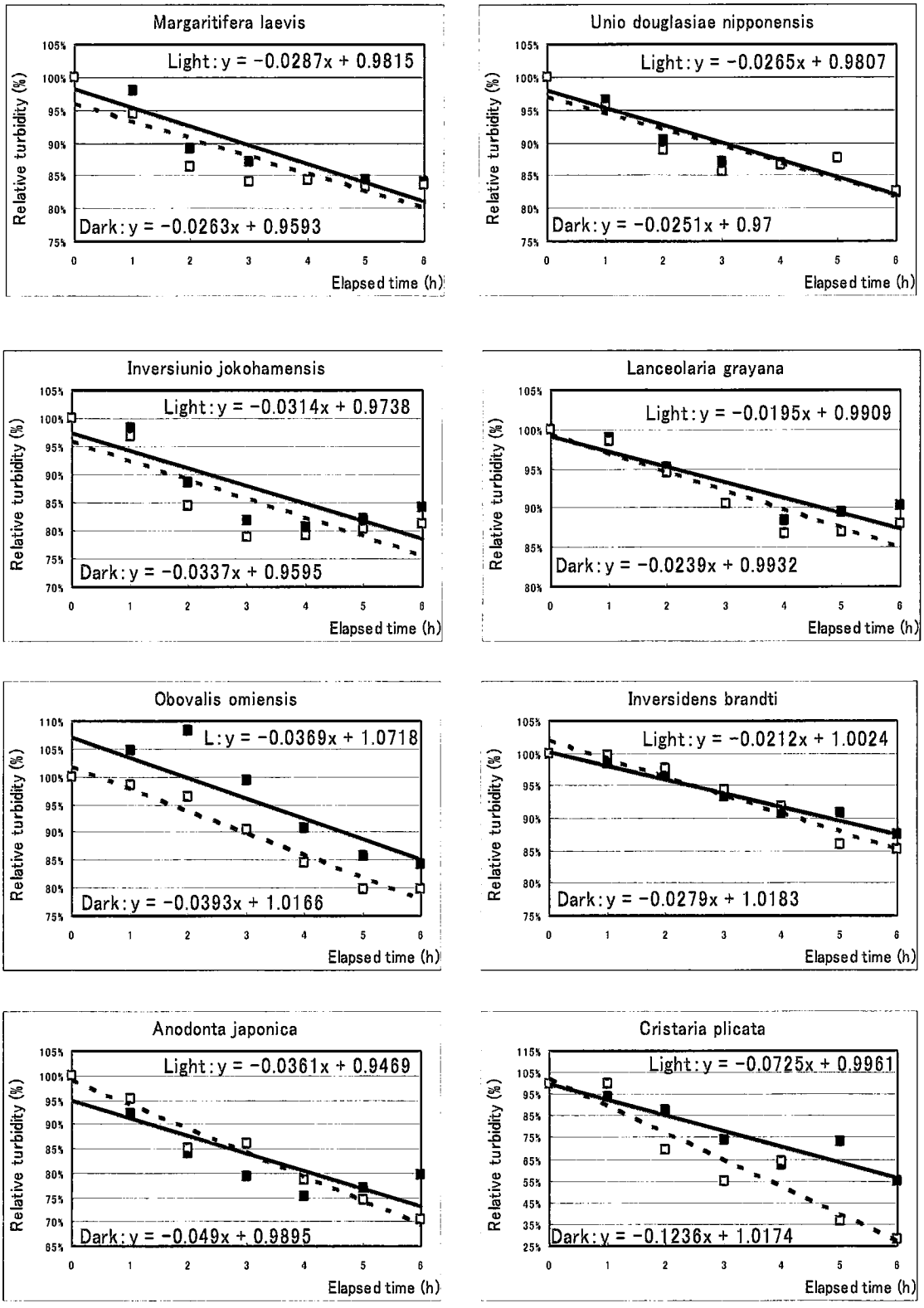


**Figure 1.** Efficacy of removal and aggregation of suspended matter per individual in each of eight unionid mussel species
Efficacy of removal and aggregation of suspended matter is shown as a percentage calculated by dividing the rate of change in turbidity for each experimental group by that of the controls. Furthermore, this was transformed into efficacy of removal and aggregation per individual by dividing by the number of mussels used in the respective experiment. Solid lines and black squares represent light conditions, and dotted lines and white squares represent dark conditions.

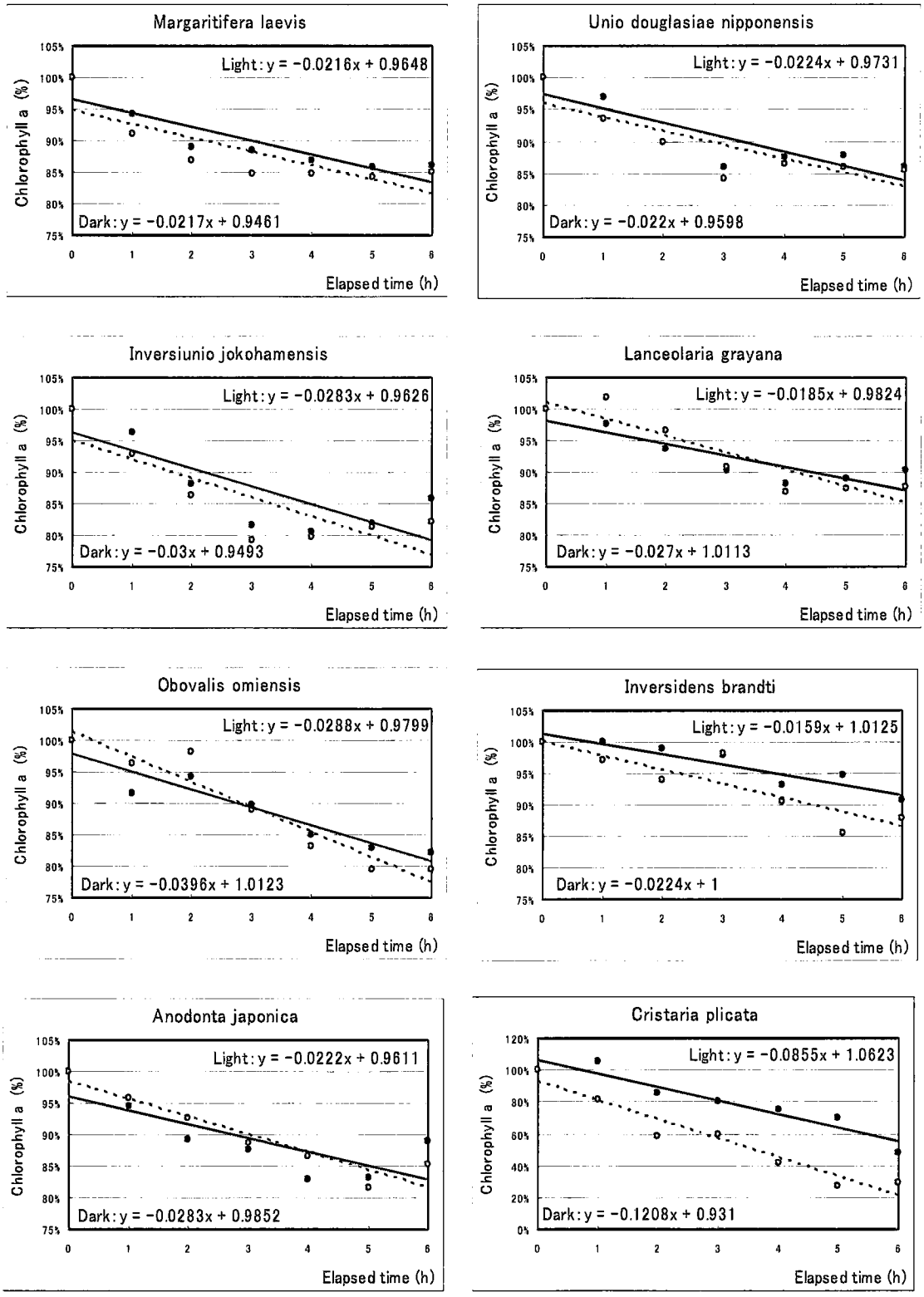


**Figure 2.** Efficacy of removal and aggregation of chlorophyll per individual in each of eight unionid mussel species
Efficacy of removal and aggregation of chlorophyll (Chl*a*) is shown as a percentage by dividing the rate of change in Chl*a* for each experimental group by that of the controls. Furthermore, this was transformed into efficacy of removal and aggregation per individual by dividing by the number of mussels used in the respective experiment. Solid lines and black squares represent light conditions, and dotted lines and white squares represent dark conditions.

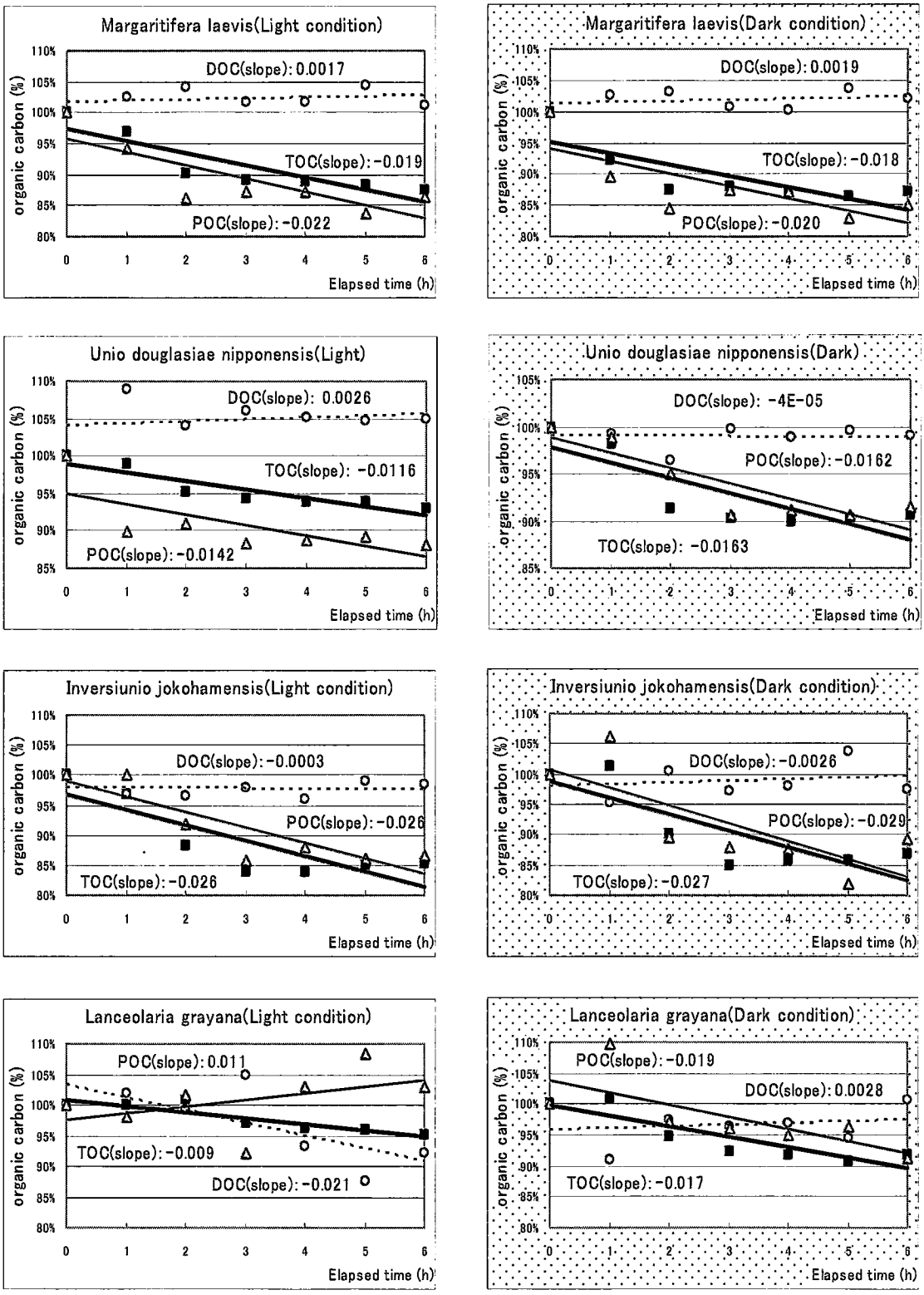


**Figure 3-1.** Efficacy of removal and aggregation of TOC, DOC and POC per individual in each of eight unionid mussel species Vertical axis indicates relative organic carbon concentration (%). The thick solid line and black squares represent changes in TOC, the dotted line and white circles represent changes in DOC and the thin sold line and white triangle represent changes in POC (POC = TOC – DOC). After showing efficacy of removal and aggregation of TOC, DOC and POC as a percentage by dividing the rates of change in TOC and DOC for each experimental group by that of the controls, this was transformed into efficacy of removal and aggregation per individual by dividing by the number of mussels used in the respective experiment.

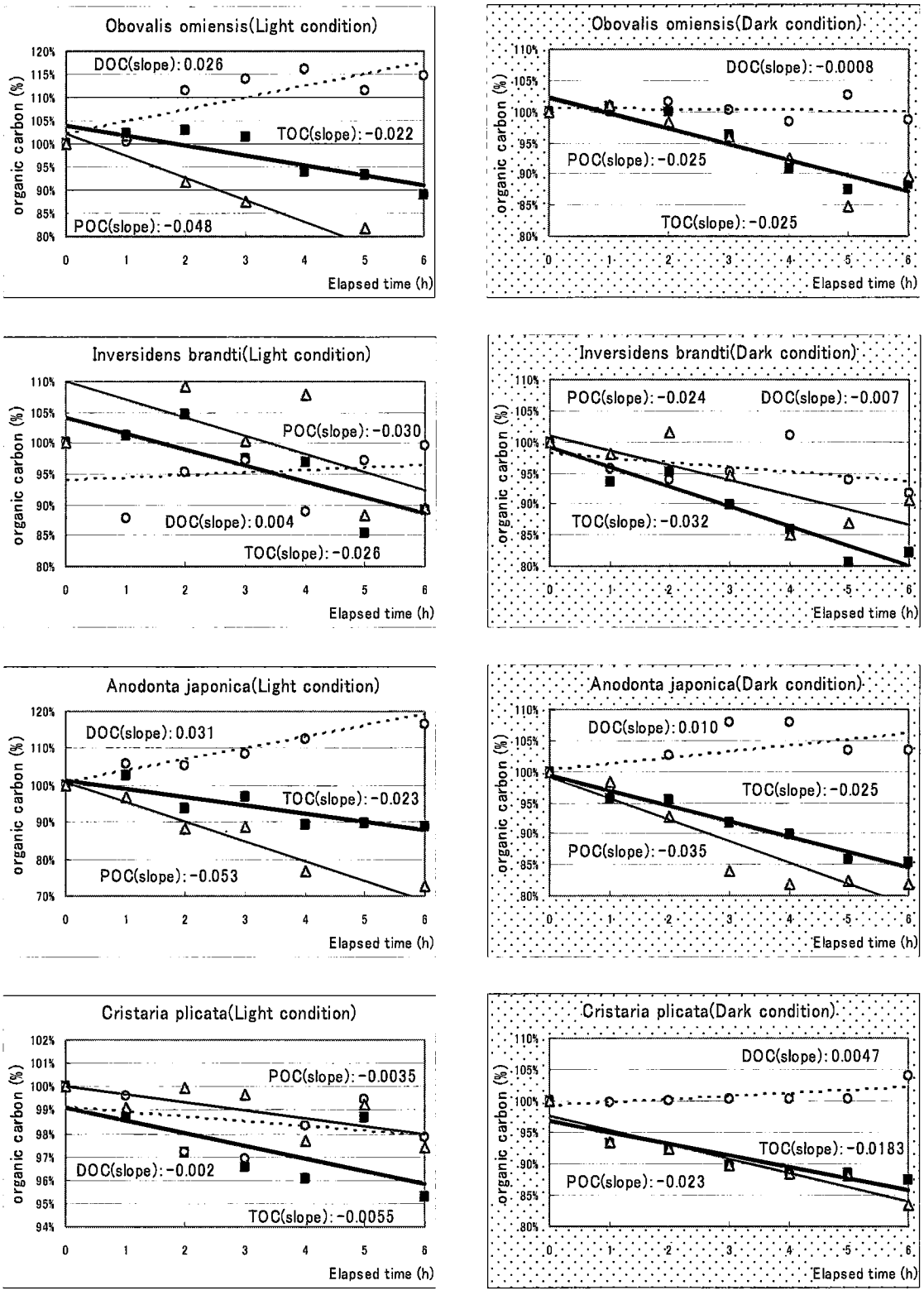


**Figure 3-2.** Efficacy of removal and aggregation of TOC, DOC and POC per individual in each of eight unionid mussel species (cont.)

Vertical axis indicates relative organic carbon concentration (%). The thick solid line and black squares represent changes in TOC, the dotted line and white circles represent changes in DOC and the thin sold line and white triangles represent changes in POC (POC = TOC – DOC). After showing efficacy of removal and aggregation of TOC, DOC and POC as a percentage by dividing the rates of change in TOC and DOC for each experimental group by that of the controls, this was transformed into efficacy of removal and aggregation per individual by dividing by the number of mussels used in the respective experiment.

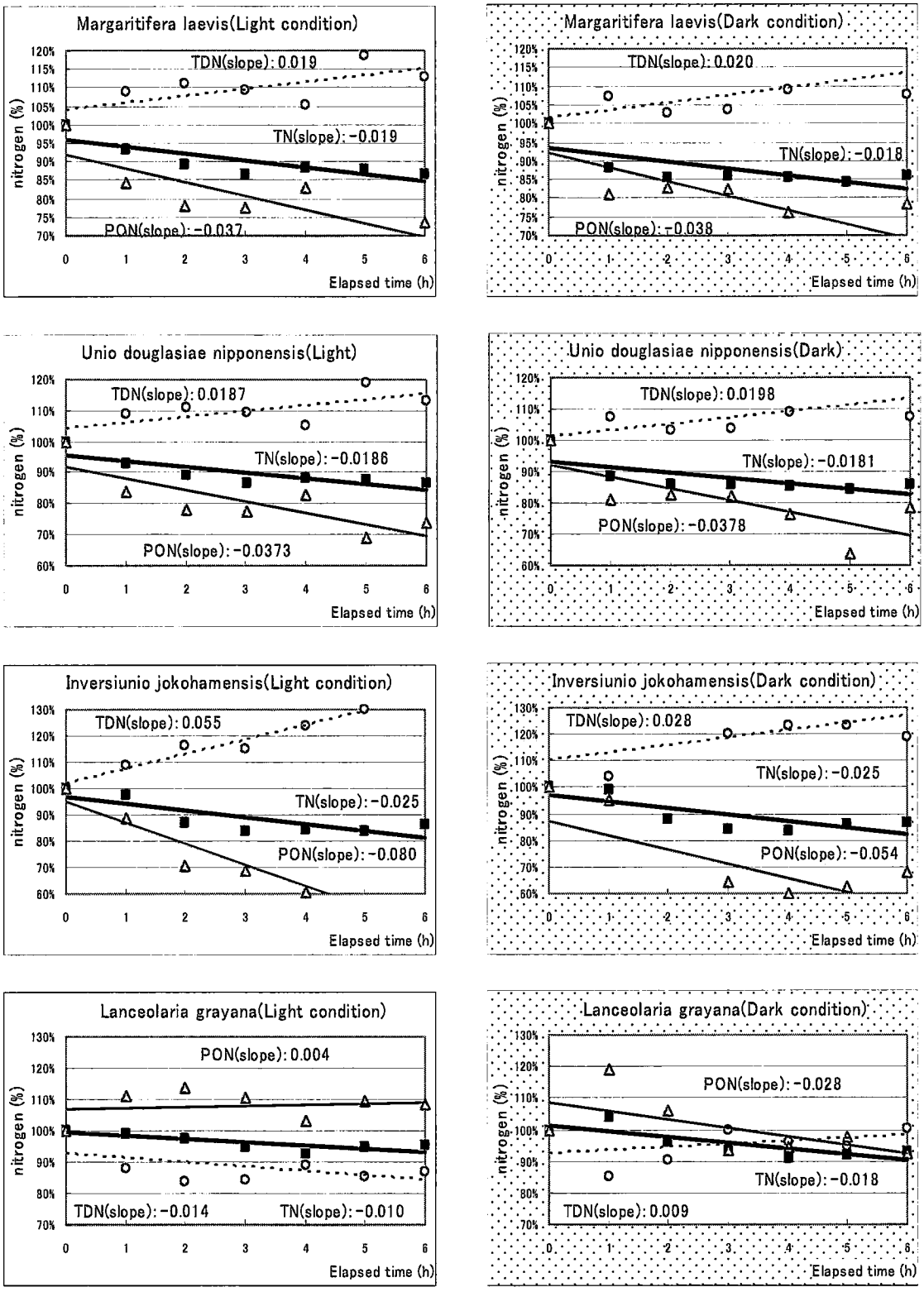


**Figure 4-1.** Efficacy of removal and aggregation of TN, TDN and PON per individual in each of eight unionid mussel species
Vertical axis indicates relative nitrogen concentration (%). The thick solid line and black squares represent changes in TN, the dotted line and white circles represent changes in TDN and the thin sold line and white triangles represent changes in PON (PON = TN – TDN). After showing efficacy of removal and aggregation of TN, TDN and PON as a percentage by dividing the rates of change in TN and TDN for each experimental group by that of the controls, this was transformed into efficacy of removal and aggregation per individual by dividing by the number of mussels used in the respective experiment.

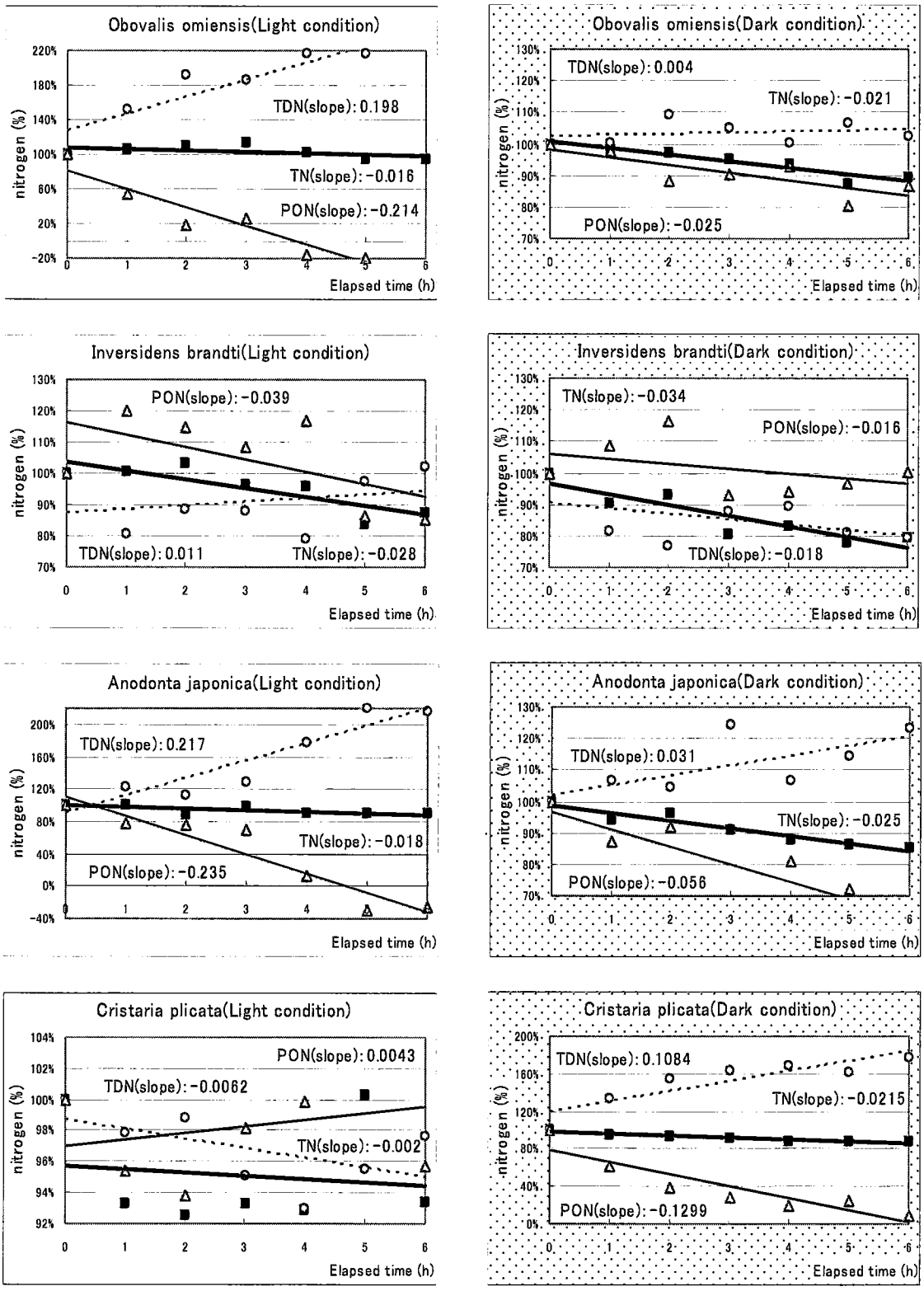


**Figure 4-2.** Efficacy of removal and aggregation of TN, TDN and PON per individual in each of eight unionid mussel species (cont.)

Vertical axis indicates relative nitrogen concentration (%). The thick solid line and black squares represent changes in TN, the dotted line and white circles represent changes in TDN, and the thin sold line and white triangles represent changes in PON (PON = TN – TDN). After showing efficacy of removal and aggregation of TN, TDN and PON as a percentage by dividing the rates of change in TN and TDN for each experimental group by that of the controls, this was transformed into efficacy of removal and aggregation per individual by dividing by the number of mussels used in the respective experiment.

## Discussion

First we will examine the effect exerted by the experimental conditions on rates of filtration and TOC and TN removal. Comparison of the filtration rate for *A. japonica*, which has been previously studied, with the results of Kryger and Rissgard (1988) reveals a lower rate for the present study. This difference may be due to measurement method. As Yamamuro (1992) has indicated, the indirect method used in the present study does not necessarily result in a 100% particle capture ratio, while in the direct method used by Kryger and Riisgard (1988), placement of a microscopic current meter adjacent to the siphons of the mussel may cause stress to the individual and thus affect the results. Mohlenberg and Riisgard (1979) reported that filtration rates more than three times greater for mussels are buried in sediment than exposed mussels. Although effects of conditions such as container size and initial turbidity (concentration of alga given as food) should be considered, conditions in the present study were regulated to avoid overcrowding and to provide sufficient food. Although there are differences between the conventional findings and values of many previous studies using simple indirect measurement methods (Yamamuro, 1992) and those of the present study, it is possible to discuss the relative interspecific differences with regard to filtration rates and TOC and TN removal.

The eight unionid mussel species examined in the present study have been shown to remove suspended matter and reduce Chl.a concentration. All eight species showed higher filtration rates under dark rather than light conditions (Table 2), clarifying that the filtration rate in these mussels increases at night. There are few studies focusing on the diurnal and nocturnal behavioral characteristics of bivalves; however, as with the nocturnal behavior of *C. Japonica* identified by Somiya *et al.* (2008) the bivalves in the present study appear to actively filter-feed at night (Table 2). Nevertheless, as these results were obtained under artificially produced light and dark conditions, further investigation is required under natural conditions to prove the nocturnal increase in filtration rate for each species.

All species used in the present study decreased TOC and TN (Figs. 3, 4; Table 2). This indicates that unionid mussels contribute to water purification by taking in TN, a principle cause of eutrophication, and TOC, which increases as a result of eutrophication. All species showed much

greater removal rates for TOC than TN (Table 2) and in many experiments, a marked decrease was seen not in dissolved matter but in particulate matter (suspended matter) (Figs. 3, 4). As absorption and digestion volumes and volume of intermittently ejected excrement (feces and pseudofeces) by the mussels are reflected in the changes in POC and DOC, and PON and TND, we can surmise the following. The reason that the DOC supply mostly neither increased nor decreased but was stable in many experiments may be because DOC was neither taken in through filtration nor ejected. Conversely, the reason for the slight increase in TDN supply over time may be that some of the mussel excrement was ejected as TDN or that TDN was eluted from the mucus excrements (pseudofeces). However, the PON removal exceeded that of the TDN increase, resulting in an overall decreasing trend in TN. These findings suggest that many of the mussels used in the present experiment remove POC and PON and excrete small amounts of TDN.

A comparison of the rates of TOC and TN removal under light and dark conditions revealed that more TOC and TN are removed under dark conditions, excluding the exception (*I. jokohamensis* and *O. omiensis*, and *I. jokohamensis)*, respectively. These findings indicate that the majority of freshwater unionid mussels, remove more TOC and TN through active nocturnal filter-feeding at night. However, it is of considerable interest that rate of TOC and TN removal per unit of wet weight does not necessarily correspond to the overall filtration rate. For example, despite the fact that the filtration rate per unit wet weight among the eight species examined in the present study was largest for *M. laevis*, TOC removal rate was greatest for *I. brandti* (Table 2). Furthermore, for *L. grayana*, in which TOC and TN removal rates per individual in both light and dark conditions were the smallest (eight in wet weight volume), filtration rate per individual was least of the eight species but conversion to per unit of wet weight places it second out of eight species in both light and dark conditions (Table 2).

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