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Changes of Lengths of Body Portions and Body Weight by Fixation with 10% Formalin in Largemouth Bass, *Micropterus salmoides*, and Bluegill, *Lepomis macrochirus*

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Abstract. Changes of lengths of body portions (total length [TL], standard length [SL], head length [HL], body depth [BD]) and body weight (BW) by fixation with 10% formalin in largemouth bass, *Micropterus salmoides*, and bluegill, *Lepomis macrochirus*, were examined to obtain basic information for scientific studies of fishes. In the largemouth bass juvenile, TL diminished to about 97% and BW increased to about 112% of the values before fixation. In the largemouth bass adult, BD diminished to about 95-96% in the larger size class (TL 200–400 mm), and BW increased to about 102–104%. In the bluegill juvenile, TL diminished to about 97%, whereas BW did not change. In the bluegill adult, although BW temporarily increased during several days just after fixation, finally, changes were not recognized at any characters. The reason why BW of the largemouth bass increase by fixation is considered that formalin solution sinks into the abdominal cavity of the specimen. The results of the present study conclude that measured values of the fixed specimens need to be adjusted for TL and BW of the largemouth bass juvenile, BD and BW of the largemouth bass adult, and TL of the bluegill juvenile.

Key words: *Micropterus salmoides*, *Lepomis macrochirus*, fixation, formalin, shrinkage.

For measurement of fish body as a scientific examination, in some cases it is instantly performed in fresh condition. However in many other cases, it is performed posteriorly for fixed or frozen preserved specimens, because it can be difficult chronologically to measure many specimens at once. For such cases, lengths of body portions and body weight after the preservation should be compared with those in flesh condition, and if they differ from each other, the measured values of the preserved specimens should be adjusted. Except for the larvae of some fish species (Kumagai & Castillo, 1980; Hay, 1984; Tucker & Chester, 1984; Jennings, 1991), such a trial has rarely been performed.

Recently in Japan, centrarchid fishes including largemouth bass, *Micropterus salmoides*, and bluegill, *Lepomis macrochirus*, which have been introduced from North America, are being vigorously biologically examined, due to social problems of exotic livings introduced into Japan (*e.g.* National Federation of Inlandwater Fisheries Cooperatives, 1992). For such scientific examinations, it can be important to grasp the changes by preservation as basic data, because such information has not been accumulated for the centrarchid fishes, except that Kuwamura (1992) examined changes of length and weight of largemouth bass by frozen preservation. Examinations of the changes by preservation in the centrarchid fishes may also be case studies for freshwater fishes because such examinations have been performed mostly for marine livings (*e.g.* Kubo & Yoshihara, 1957). Therefore in the present study, chronological changes of lengths of body portions and body weight by fixation with 10% formalin, which is the most popular preservative method, were

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examined to estimate the values before fixation in the fixed specimens.

Materials and Methods

Largemouth bass

Specimens of juveniles and adults (including fingerlings) were used for the experiments. All specimens were from Hachiman Reservoir, Kagawa Prefecture (Yokogawa, 1992*a*), the juvenile specimens were collected on May 24, 1991 with a scoop net, and the adult specimens were caught on January 17, 1991, with a surrounding net, when cultured fish in the reservoir were harvested. The specimens were instantly measured for the lengths of the body portions and body weight, and they were immediately soaked into 10% formalin solution. Thereafter, the lengths and weight were periodically remeasured to grasp their chronological changes. The measured characters were two (total length and body weight) for the juveniles, and five (total length, standard length, head length, body depth and body weight) for the adults. Methods of the measurements followed Hubbs & Lagler (1970).

Total lengths before fixation are, 19.8–25.4 mm (16 individuals) in the juveniles, 151.0–398.0 mm in the adults. The adult specimens were classified into three size classes of TL 100–200 mm (7 individuals), TL 200–300 mm (11 individuals) and TL 200–400 mm (8 individuals). For calculation of changing rates, individual changing rate was calculated at every remeasured time for each character, average value calculated for each size class was regarded as the changing rate of the class. Further, t-test between the changing rate at every remeasured time (expressed as a percentage of the original value) and that before fixation (regarded as 100%) was performed to evaluate statistical significance between them.

Bluegill

Specimens of juveniles and adults (including fingerlings) were used for the experiments. The juveniles were from Manno Reservoir, Kagawa Prefecture (Yokogawa, 1992*a*) on July 10, 1991, collected with a specially designed collecting net (Yokogawa, 1992*b*). While the adults were from Hachiman Reservoir, caught together with the largemouth bass specimens used for the present study. Total lengths before fixation are, 10.2–14.9 mm (6 individuals) in the juveniles, 96.0–199.0 mm in the adults. The adult specimens were classified into three size classes of TL 80–120 mm (7 individuals), TL 120–160 mm (12 individuals) and TL 160–200 mm (5 individuals). Methods of the experiments and calculation were the same as those for the largemouth bass earlier mentioned.

Results

Largemouth bass (*juvenile*)

Chronological changes of total length (TL) and body weight (BW) up to 45 days after fixation were illustrated in Fig. 1. TL instantly diminished after fixation, then dropped down to less than 97%. Thereafter TL has been almost stable within a range of 96–98%, and significant differences from the original value were occasionally recognized (Fig. 1). While BW instantly increased after fixation, being more than 115%. Thereafter, BW has been almost stable within a range of 111–114%, and all values after fixation showed significant differences from the original value (Fig. 1). Thus, TL of the largemouth bass juvenile was regarded to diminish to about 97% by fixation, while its BW was regarded to increase to approximately 112% by fixation.

Largemouth bass (*adult*)

Chronological changes of total length (TL), standard length (SL), head length (HL), body depth (BD) and body weight (BW) of largemouth bass adults (divided into three size classes) up to 171 days after fixation were illustrated in Fig. 2.

TL was almost stable after fixation in any size class, although it sifted toward somewhat greater than the original value in the 200–300 mm class, and sifted toward somewhat smaller than the original value in the 300–400 mm class. However, any

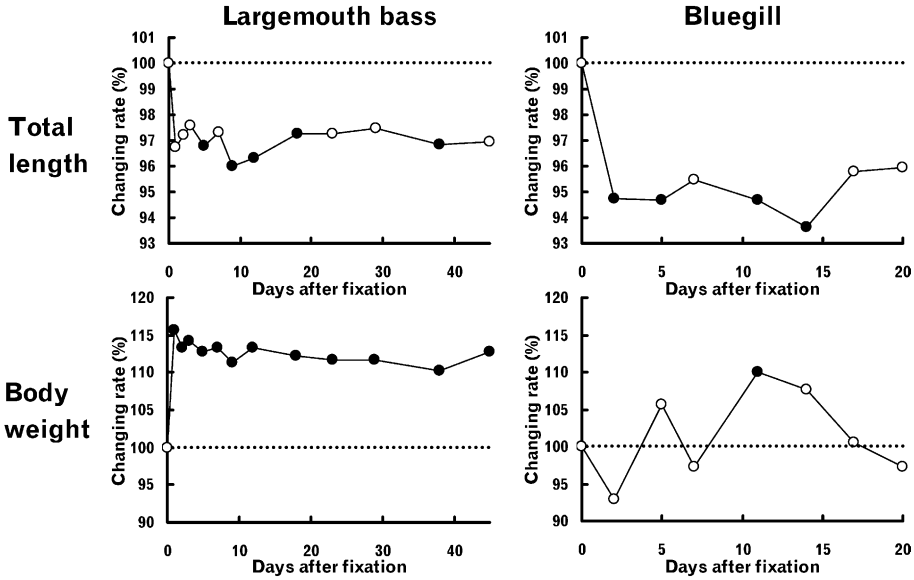

Fig. 1. Chronological changes of total length (TL) and body weight (BW) of largemouth bass and bluegill juveniles up to 45 and 20 days after fixation, respectively. Broken lines indicate the original value (100%) levels. Open circles — values in which significant differences from the original values were not recognized by t-tests. Dark circles — values in which significant differences at less than 5% level from the original values were recognized by *t*-tests.

significant differences from the original value were not recognized at any remeasured values in any size class. Therefore, TL of the largemouth bass adult was regarded as unchanged by fixation.

SL also was almost stable after fixation in any size class, although it sifted toward somewhat greater than the original value in all the classes. However, any significant differences from the original value were not recognized at any remeasured values in any size class. Therefore, SL of the largemouth bass adult was also regarded as unchanged by fixation.

HL in the 100–200 mm and 200–300 mm classes were almost stable after fixation, all remeasured values showed no significant differences from the original value. While that in the 300–400 mm class increased after fixation, indicated values about 102–103%, significant differences from the original value were recognized at some remeasured values until 60 days after fixation. However thereafter, it converged in the value of about 101%, no significant differences from the original value were recognized after 60 days after fixation. These matters suggest that HL of the largemouth bass adult also is regarded as unchanged by fixation.

BD in the 100–200 mm class has been almost stable after fixation, showing no significant differences from the original value. While that of 200–300 mm and 300–400 mm classes diminished after fixation, converging in the values of about 96% and 95% in the 200–300 mm and 300-400 mm classes, respectively. And some and many significant differences from the original value were recognized in the 200–300 mm and 300–400 mm classes, respectively. Thus for BD of the largemouth bass adult, it is regarded as unchanged by fixation in the smaller (100–200 mm) class, and is regarded to diminish to 95–96% by fixation in the larger (200–300 mm and 300–400 mm) classes. BW increased in all the size classes, although the changing patterns differed from one another. In the 100–200 mm class, it instantly increased to the value of approximately 105%, subsequently dropped rapidly, thereafter repeated up and down, consequently converged to the value of

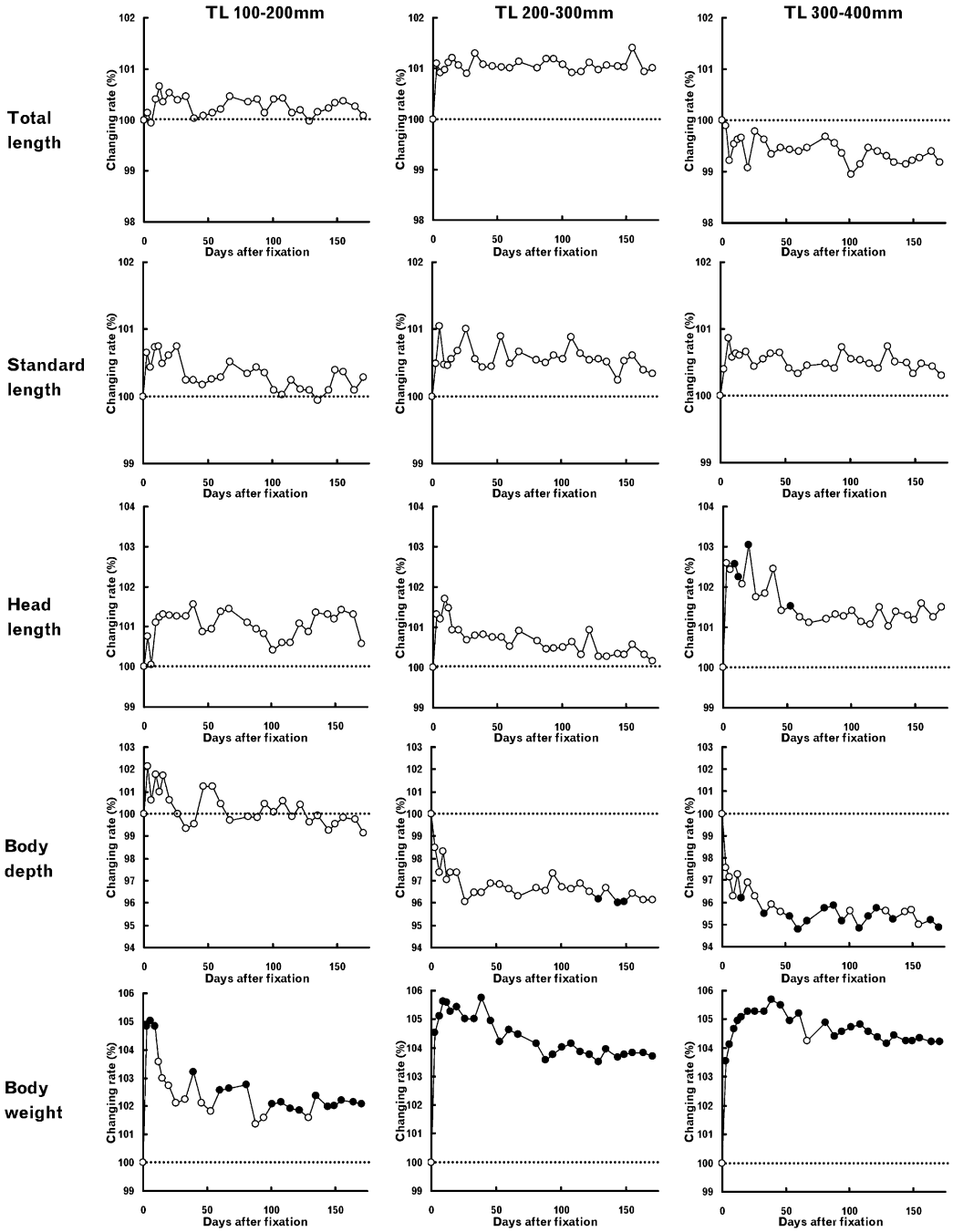

Fig. 2. Chronological changes of total length (TL), standard length (SL), head length (HL), body depth (BD) and body weight (BW) of largemouth bass adults (divided into three size classes) up to 171 days after fixation. Broken lines indicate the original value (100%) levels. Open circles — values in which significant differences from the original values were not recognized by t-tests. Dark circles — values in which significant differences at less than 5% level from the original values were recognized by *t*-tests.

about 102%. While in the 200–300 mm and 300–400 mm classes, BW increased after fixation and reached near to 106%, thereafter it gradually diminished and converged to the value of about 104%. In all the classes, all or most of remeasured values showed significant differences from the original value. For BW of the largemouth bass adult, it is regarded to increase by fixation to 102% and 104% in the smaller (100–200 mm) class and the larger (200–300 mm and 300–400 mm) classes, respectively.

Bluegill (*juvenile*)

Chronological changes of total length (TL) and body weight (BW) until 20 days after fixation were illustrated in Fig. 1. TL instantly diminished after fixation, then dropped down to less than 95%. Thereafter, TL has been almost stable within a range of 93–96%, and significant differences from the original value were occasionally recognized (Fig. 1). While BW changed almost randomly, it finished in very close value to 100% (Fig. 1). Although only one significant difference was recognized during the experiment, it would be regarded to occur by chance during the random fluctuation. Thus, TL of the bluegill juvenile was regarded to diminish to about 97% by fixation, while its BW was regarded as unchanged by fixation.

Bluegill (*adult*)

Chronological changes of total length (TL), standard length (SL), head length (HL), body depth (BD) and body weight (BW) of bluegill adults (divided into three size classes) up to 171 days after fixation were illustrated in Fig. 3.

TL in the 90–120 mm class inclined to diminish gradually, reaching below 99% at the end of the experiment. However, significant differences from the original value were not recognized at any remeasured values during the experiment. While that in the 120–160 mm and 160–200 mm classes, it was very stable after fixation, indicating a fluctuation around 100%. Also, any significant differences from the original value were not recognized at any remeasured values in the two size classes. Therefore, TL of the bluegill adult was regarded as unchanged by fixation.

SL was almost stable after fixation in any size class, although it sifted toward somewhat greater than the original value in the 90–120 mm class. However, significant differences from the original value were not recognized at any remeasured values during the experiment in the class. In the remaining two size classes, SL both indicated fluctuations around 100%. Although only one significant difference was recognized in the 120–160 mm class in an earlier day after fixation, SL in the class finally converged in almost 100%. Therefore, SL of the bluegill adult was regarded not to change by fixation.

HL in the three size classes instantly increased to about 102% after fixation, thereafter it sifted toward below 100% in the 90–120 mm and 120–160 classes. In the two size classes, all remeasured values showed no significant differences from the original value. While in the 160–200 mm class, HL consequently converged near to 100%, and significant differences were recognized at two remeasured values in later days after fixation. However, these significant differences would be ignored because HL of the class well converged in about 100% at last. Thus, HL of the bluegill adult was regarded as unchanged by fixation.

BD in the three size classes indicated fluctuations mostly within zones below 100%. Although only one significant difference was recognized in the 120–160 mm class in an earlier day after fixation, BD inclined to approach 100% levels in all the classes. Thus, BD of the bluegill adult was regarded as unchanged by fixation.

BW instantly increased in all the size classes, temporarily reached 103–104% in the 90–120 mm and 120–160 mm classes. Consequently it rapidly or gradually diminished, inclined to approach 100% levels in all the classes. Although a few significant differences were recognized in the 120–160 mm and 160–200 mm classes in earlier several days just after fixation, when BW temporarily increased, BW finally converged in near to 100% in the classes. Therefore, it is concluded that BW of the bluegill adult once

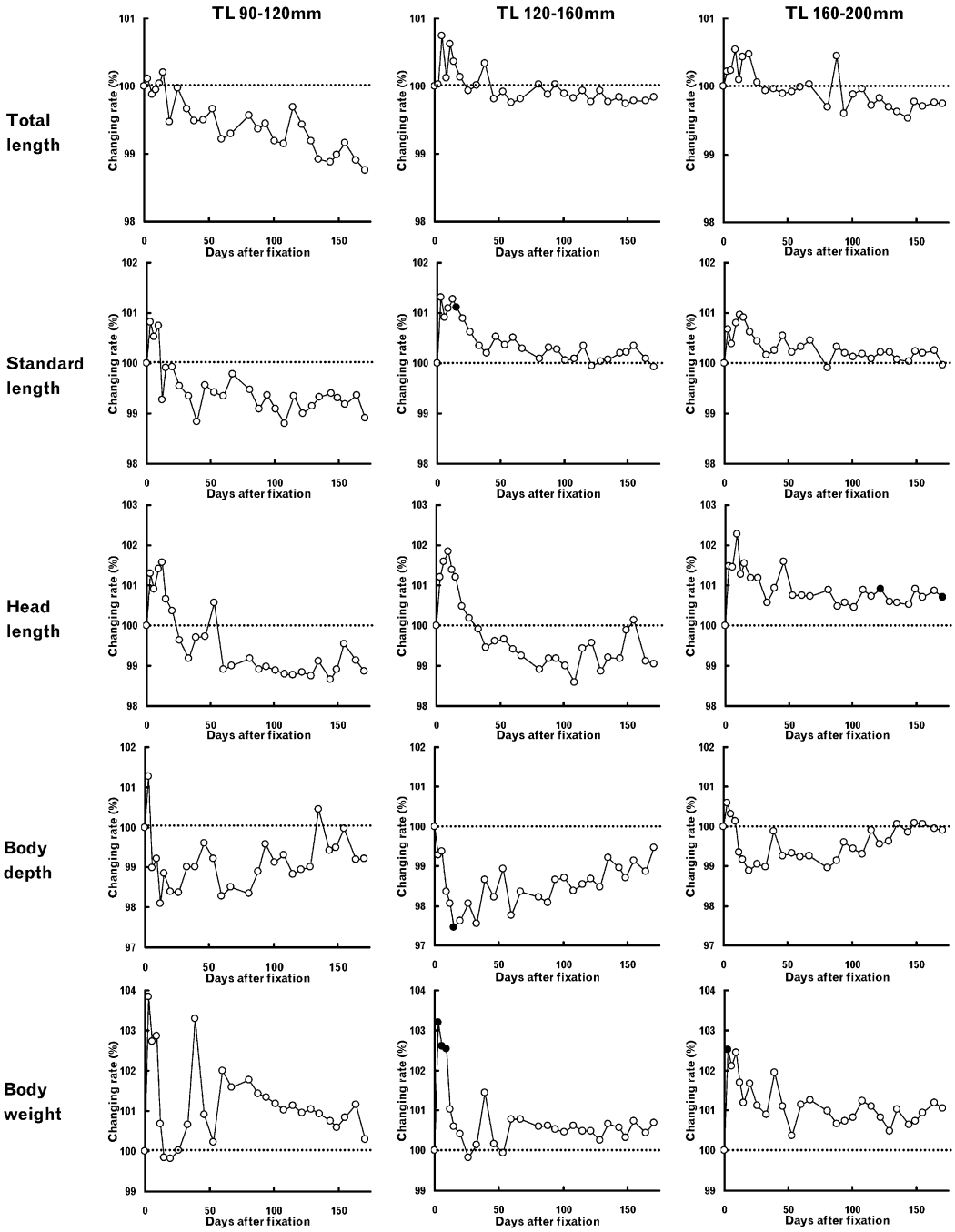

Fig. 3. Chronological changes of total length (TL), standard length (SL), head length (HL), body depth (BD) and body weight (BW) of bluegill adults (divided into three size classes) up to 171 days after fixation. Broken lines indicate the original value (100%) levels. Open circles — values in which significant differences from the original values were not recognized by *t*-tests. Dark circles — values in which significant differences at less than 5% level from the original values were recognized by *t*-tests.

increased temporarily by fixation, it converged to the level before fixation during long-term preservation.

Discussion

The present study revealed that values of some characters of the largemouth bass and bluegill significantly changed by fixation with 10% formalin.

The significant changes are summarized as follows.

1. TL of the largemouth bass juvenile diminishes approximately to 97% of the value before fixation.
2. BW of the largemouth bass juvenile increases approximately to 112% of the value before fixation.
3. BD of the largemouth bass adult diminishes approximately to 96% and 95% of the values before fixation in the 200–300 mm and 300–400 mm classes, respectively. While it does not change in the 100–200 mm class.
4. BW of the largemouth bass adult increases approximately to 102% and 104% of the values before fixation in the 100–200 mm and 200–400 mm classes, respectively. The value of 104% may also be applied for larger specimens than 400 mm.
5. TL of the bluegill juvenile diminishes approximately to 97% of the value before fixation.

Therefore, the measured values of these characters of the fixed specimens need to be adjusted to those before fixation. For the adjusted calculation, each measured value of a character should be multiplied by a reciprocal number of the changing rate of the character.

TL of the largemouth bass and bluegill juveniles diminished by fixation, this phenomenon has been commonly observed in larvae and juveniles of various fish (including cyclostome) species such as milkfish, *Chanos chanos* (Kumagai & Castillo, 1980), Pacific herring, *Clupea harengus pallasi* (Hay, 1984), southern flounder, *Paralichthys lethostigma* (Tucker & Chester, 1984), European sea bass, *Dicentrarchus labrax* (Jennings, 1991), and sea lamprey, *Petromyzon marinus* (Morkert & Bergstedt, 1990). Such diminution of length in larvae and juveniles is regarded as shrinkage occurred by an osmotic effect of formalin.

Although the shrinkage by formalin fixation occurs not only in larvae and juveniles but also in adult of some fish species (Kubo & Yoshihara, 1957; Sato, 1972), the adult specimens of the largemouth bass and bluegill do not shrink in TL and SL (Figs. 2, 3). This suggests that vertebrae and muscular systems of their trunks are so tightly developed that the osmotic effect hardly occurs in the adults of the two species. While, Kuwamura (1992) reported that length of largemouth bass adult significantly diminished by a frozen preservation. Although an explanation as to why the length is lost is uncertain, that fact should be noted when a biological study is performed.

BD of the largemouth bass adult diminished by fixation in the 200–300 mm and 300–400 mm classes, this would also be regarded as a kind of shrinkage. In case of the largemouth bass larger specimens, the abdomen tends to be bloated and sag, it subsequently becomes somewhat tight by fixation, consequently BD would diminish. This phenomenon applies only to the larger size of largemouth bass, but does not apply to the smaller size specimens (100–200 mm), in which the abdomen is not bloated (Fig. 2). It also does not apply to the bluegill (Fig. 3).

The fact that BW in the largemouth bass significantly increases by fixation is particularly remarkable, because BW is frequently used for calculations of condition factor, length-weight relationship and so on.

The reason why BW of the largemouth bass increase by fixation is considered. In ecological studies for the largemouth bass, the specimens are often anatomized to examine stomach contents, gonads and so on. When the abdomen of a fixed specimen of the largemouth bass is cut and open, a considerable amount of fluid, which is regarded as formalin solution, flows out of the abdominal cavity. That fluid may cause the increment of BW. Therefore, the abdomens of the specimens examined in the present study were cut and opened on the final day of the experiments, the fluid in the abdominal cavity was removed, and BW of the fluid ridded specimens were weighed.

Table 1 shows results of such a trial, together

Table 1. Body weights (% of original value) at the final remeasurement and those when abdominal fluid was ridded, together with results of *t*-tests between the original values and those values in largemouth bass and bluegill.

Largemouth bass			Bluegill		
	BW (% of original value) at the final remeasurement	BW (% of original value) when abdominal fluid was ridded		BW (% of original value) at the final remeasurement	BW (% of original value) when abdominal fluid was ridded
TL 100–200 mm			TL 90–120 mm		
Average	102.1	97.1	Average	100.3	93.1
t	2.851 *	2.499	*t*	0.150	2.921 *
TL 200–300 mm			TL 120–160 mm		
Average	103.7	100.5	Average	100.7	96.1
t	4.226 **	0.686	*t*	0.817	3.009 *
TL 300–400 mm			TL 160–200 mm		
Average	104.2	101.1	Average	101.0	99.0
t	9.956 ***	1.742	*t*	0.739	0.439

* Significant at 5% level. ** Significant at 1% level. *** Significant at 0.1% level.

with those performed for the bluegill specimens used for the present study (This trial has not been performed for the juveniles of the largemouth bass and the bluegill). On the final day of the experiments, the average BW changing rates of the largemouth bass specimens were 102.1–104.2%, being significantly larger than the values before fixation (100%) in all the size classes. While those of the fluid ridded specimens in the 200–300 mm and 300–400 mm size classes were 100.5–101.1%, showing no significant differences from the values before fixation (Table 1). These matters indicated that the fluid in the abdominal cavity, which sank into after fixation, caused the increment of BW.

While in the 100–200 mm size class, the average BW changing rate of the fluid ridded specimens resulted in 97.1%, being significantly smaller than the value before fixation. This phenomenon infers that some amount of fluid existed in the abdominal cavity before fixation, and it was taken away together with the fluid (formalin solution) sank into the cavity after fixation, consequently resulting in the significant BW smallness of the fluid ridded specimens. The pre-existing body fluid might be common to any size of specimens; however, its amount is relatively less in the larger specimen's body that it would hardly affect the BW even though it was removed together with the sank fluid.

In case of the largemouth bass juvenile, BW considerably increased after fixation, fell into about 112% (Fig. 1), this may concern with such a theory.

That is, even a little amount of fluid in the cavity would sensitively affect BW because original BW of the individual in that stage is so little.

Table 1 also shows results of the trial for the bluegill specimens. As for the bluegill, the average BW changing rate at the final measurement did not significantly differ from the values before fixation (100%) in all size classes.

While the average BW changing rates of the abdominal fluid ridded specimens were significantly smaller than the value before fixation in the smaller (90–120 mm and 120–160 mm) classes. These matters can be explained with the theory hypothesized for the largemouth bass.

On the other hand, an explanation as to how the formalin solution sinks into the abdominal cavity is uncertain. But it may be likely to regard that the formalin solution sinks into the cavity via abdominal skin. The phenomenon that BW increases by fixation has been reported for some other fishes such as Japanese sardine, *Sardinops melanostictus* (Kubo & Yoshihara, 1957), rainbow trout, *Oncorhynchus mykiss* (Sato, 1972), and yellow perch, *Perca flavescens* (Stobo, 1972). Stobo (1972) suggested that the osmotic processes involved in the weight gain, it may be possible. But anyway, the fact that BW increases by fixation itself would be more important

for the practical research than its mechanism.

The present study revealed that values of some characters in the largemouth bass and bluegill significantly changed by fixation with formalin.

Such examinations necessitate as basic data for measurements of fixed fish specimens. Since characters which significantly change and their changing patterns are different from one another by species and growth stage, they should be examined whenever biological studies for a fish is performed.

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