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A test of the ash-free dry weight technique on the developmental stages of *Patiriella* spp. (Echinodermata: Asteroidea)

Abstract—Determination of the ash-free dry weight (AFDW) of marine specimens requires samples to be rinsed, soaked, and centrifuged. Problems associated with this technique were examined with the developmental stages of seastar species (*Patiriella*) with different modes of development. The influence of three rinsing solutions (ammonium formate [AF], filtered seawater [FSW], and reverse osmosis water [RO]) was assessed. The hypothesis that the AFDW technique is a measure of organic material was addressed by drying inorganic salts. Developmental stages of *Patiriella calcar* rinsed in FSW were twice as heavy as those rinsed in RO or AF, indicating that samples should be rinsed in RO or AF before weighing. Soaking treatments had a significant effect on the AFDW of samples of *P. calcar* (planktonic developer), indicating that the rinsing period should be brief. Zygotes of *Patiriella regularis* (planktonic developer) were significantly heavier than ova or gastrulae, regardless of treatment. In contrast, there were no significant differences in the AFDW of any stages or treatments of *Patiriella exigua* (benthic developer). This may be due to the presence of a modified fertilization envelope, which protects these benthic embryos. Inorganic salts with water of crystallization and FSW lost 20–75% and 14% of their dry weight, respectively, after ashing. We propose that salt ions may retain water, which does not evaporate during drying but is lost during ashing, resulting in the overestimation of sample AFDW. If a similar process occurs in the developmen-

tal stages of marine invertebrates, changes in the intracellular ionic composition through development may result in inaccurate estimates of biomass.

Questions about the energetics of development are typically addressed through biomass estimates, respiration, caloric content, or carbon–hydrogen–nitrogen analyses (Gnaiger and Bitterlich 1984; McEdward and Coulter 1987; Jaeckle and Manahan 1989). Of these, the ash-free dry weight (AFDW) technique is most commonly used to measure biomass of marine invertebrate larvae (Jaeckle and Manahan 1989; Shilling and Manahan 1990; Hoegh-Guldberg 1994; Hoegh-Guldberg and Emlet 1996; Moreno and Hoegh-Guldberg 1999). In this technique, salts attached to the samples are rinsed away with distilled water (Dobberteen and Pechenik 1987; McEdward and Coulter 1987; McEdward et al. 1988; Hoegh-Guldberg and Emlet 1996; Moreno and Hoegh-Guldberg 1999) or ammonium formate (Jaeckle and Manahan 1989; Shilling and Manahan 1990; Hoegh-Guldberg 1994). Samples are dried (≈80°C) for approximately 10 d, weighed (dry weight), then ashed (≈480°C) for 6 h and weighed again (ash weight). The difference between these two weights, the AFDW, is considered to represent the organic biomass that was burnt. Even though the handling steps required in the AFDW technique could result in the damage of fragile developmental stages, we know of no study that has tested for possible artifacts associated with the steps of this technique.

Common inconsistencies associated with the AFDW technique include: (1) the increase in weight between the ova and gastrulae (nonfeeding) stages reported for several invertebrate taxa (Manahan et al. 1990; Shilling and Manahan 1990; Shilling and Bosch 1994), (2) the energetic imbalance observed when the energy lost through respiration is higher than that lost as biomass (Dawirs 1983; Anger 1986; Jaeckle and Manahan 1989; Shilling and Bosch 1994; Moreno and Hoegh-Guldberg 1999), and (3) the ''remainder fraction,'' when the sum of the biochemical constituents (lipid, carbohydrate, and protein) do not add up to the AFDW (Gnaiger and Bitterlich 1984; Lucas and Crisp 1987; Jaeckle 1995). Although several studies suggest that methodological limitations of the AFDW technique are responsible for these anomalies, the technique has not been examined for potential artifacts. Furthermore, ammonium formate or water is used to rinse specimens to remove sea salts from the outside of ova and embryos. Theoretically, however, inorganic salts should not affect the weights of the ova or embryos because their melting and evaporation points are much higher (>700°C) than temperatures used to dry or ash them. Thus, salts present after drying should still be present after ashing and should not influence the weight of the sample. Nevertheless, the removal of salts from the surface of ova, embryos, and larvae is considered an important step in the procedure. The procedure required to remove these salts, such as centrifugation, along with the time spent in the rinsing solution, increases the possibility of introducing artifacts through the damage of delicate developmental stages. Moreover, if salts on the outside of ova, embryos, and larvae have an effect on weight estimation, the presence and possible effects of intracellular ions on these stages should also be considered.

In this study, we explored potential artifacts associated with the AFDW technique through a series of experiments with the early developmental stages of three species of the seastar genus *Patiriella*. *Patiriella regularis* has planktotrophic development (170-μm-diameter ova; AFDW and energetic content = 0.42–0.60 μg and 14.4 mJ, respectively). *Patiriella calcar* has planktonic lecithotrophic development (415-μm-diameter ova; AFDW and energetic content = 11.11–14.58 μg and 410 mJ, respectively), and *Patiriella exigua* has benthic lecithotrophic development (390-μm-diameter ova; AFDW and energetic content = 8.72–14.06 μg and 445 mJ, respectively) (Byrne et al. 1999; Moreno and Hoegh-Guldberg 1999). The use of three congeneric species with eggs differing in size and biochemical profile provided the opportunity to assess how the AFDW procedure may affect different egg types and developmental stages. Finally, the hypothesis that the AFDW technique burns only organic material was addressed by processing inorganic salt solutions and measuring changes in weight.

Spawning and fertilization—*P. regularis* was collected in Hobart, Tasmania (42°50′S, 147°15′E). *P. calcar* and *P. exigua* were collected in Sydney, New South Wales (33°54′S, 151°17′E). Ova were obtained through use of the ovulatory hormone 1-methyladenine (10^{-3} M) in filtered seawater (FSW). Ova from 5–10 females were pooled to ensure there were sufficient numbers for adequate replication then were fertilized with sperm from two to five males. After 10 min, the excess sperm was rinsed off, and ova were checked for the presence of a fertilization envelope. Each fertilization is referred to as a batch. Embryos were reared as described by Moreno and Hoegh-Guldberg (1999).

Biomass estimates—AFDW measurements were obtained for ova, zygotes (10 min after fertilization), and gastrulae of all three species. Three beakers with three replicates per beaker for each stage were sampled. Developmental stages (200–500 for *P. regularis* and 20–40 for *P. calcar* and *P. exigua*) were rinsed once in reverse osmosis water (RO) and stored (−20°C) until analyzed. Samples were defrosted immediately prior to analysis and transferred into aluminum dishes that were preashed at 480°C for 6 h. Samples were dried at 80°C for 10 d, a time at which a constant mass was reached. They were then stored in a desiccator containing silica gel to ensure they remained dry and were weighed to the nearest microgram on a Sartorius M2P Microbalance. The microbalance was situated in an air-conditioned room to ensure that samples would not hydrate due to variable humidity. Aluminum dishes containing silica gel were placed inside the microbalance to ensure any moisture present would not cause hydration of the sample. After dry weights were measured, the samples were ashed at 480°C for 6 h and weighed again. AFDW was calculated by subtracting the ash weight from the total dry weight.

Centrifugation and soaking time experiments—Experiments were conducted to investigate the effect of two steps in the AFDW technique, centrifugation and soaking, on the

Table 1. Summary of tests conducted on the developmental stages of *P. regularis*, *P. calcar*, and *P. exigua* to determine the effect on their weight from various handling steps of the AFDW technique. zy, zygote; gast, hatched gastrula; NS, not significant.

Species	Treatment	SNK	*N*	*F*	*P*
P. regularis	Centrifugation	zy > ova = gast	2	17.85	<0.0001
P. regularis	Centrifugation	zy 10 s > zy 3–4 s	6	2.39	<0.048
P. regularis	Soaking	zy > ova = gast	2	7.75	<0.0016
P. calcar	All	ova > gast	1	10.29	<0.0075
P. calcar	Soaking	ova and gast 2 min = ova & gast 3–4 s > ova & gast 30 s	2	4.48	<0.0352
P. calcar	Rinsing	ova FSW > RO = AF	8	2.50	<0.0246
P. calcar	Rinsing	gast FSW > RO = AF	2	520.1	<0.0001
P. exigua	All treatments	NS			NS

weights of ova, zygotes, and gastrulae of *P. regularis* (n = one batch), *P. calcar* (n = one batch), and *P. exigua* (n = three batches). Three centrifugation times were used: 3–4, 10, and 20 s. Three single soaking times were tested in a separate set of trials with the same stages from the same batches: 4–10 s (i.e., the minimum amount of time it took to drain the RO after rinsing), 30 s, and 2 min. The weight data were checked for homogeneity of variances (Cochran's test), and a two-way analysis of variance (ANOVA) was performed on balanced data sets with stage and centrifugation or soaking times as fixed orthogonal factors (GMAV5 statistical package; Underwood and Chapman).

Multiple rinses experiment—The effect of multiple rinses in RO on the ova, zygotes, and unhatched gastrulae of *P. regularis* (n = one batch) was investigated. The samples were rinsed with RO once, twice, or three times and drained after each rinse. Each rinse lasted between 4 and 10 s. The weight data were checked for homogeneity of variances, and comparisons were made with a one- or two-way ANOVA on balanced data sets. Additional samples were taken for visual estimates of ova damage.

Effects of rinsing solutions—The effect of rinsing with various solutions on ova, hatched gastrulae, and early brachiolaria larvae (4 d old) of *P. calcar* (n = five batches) was tested. Three rinsing solutions were used: FSW, 3.4% AF, and RO. The data were square root transformed to achieve homogeneity of variances, and a two-way ANOVA was performed with rinsing solution as a fixed orthogonal factor and stage as a fixed nested factor. In addition, the slopes of the regressions of the AFDW of the developmental stages were compared with an analysis of covariance (JMP vers. 3.1.6; SAS), with time and treatment as covariates, to determine the effect of each rinsing solution on the weight of the stages.

Effects of drying and ashing on the weight of inorganic salts—Solutions of salts used to make artificial seawater were also subjected to the AFDW technique. Five inorganic salts used in the MBL artificial seawater formula (Cavanaugh 1975) were diluted in Milli-Q water; these included; KCl, NaCl, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, and $NaHCO_3$. A 400-μl sample of a 6% solution of each salt was frozen (−20°C), freeze dried, dried at 80°C for 10 d and weighed, and then ashed at 480°C and weighed again. These samples had to be lyophilized before drying because through capillary action, the water spilled out of the dishes if they were dried directly, resulting in loss of sample. Two experiments were conducted to weigh each salt, except for $CaCl_2 \cdot 2H_2O$, for which the second sample was lost. Each experiment consisted of six replicates per salt. Additional aluminum dishes were processed with FSW and controls (no solution). Individual salts were compared before (dry weight) and after ashing (ash weight) to determine change in weight. It was hypothesized that there would be no difference between dry and ashed samples. The sublimation point for all of these salts is well above the ashing temperature of 480°C used in this experiment (Stecher 1968); therefore, no loss of salts was expected.

Centrifugation and soaking time experiments: P. regularis—The AFDWs of the three developmental stages were significantly different regardless of treatment in the centrifugation experiments. Zygotes were heavier than ova or gastrulae ($F_{2,18} = 17.85$, $P < 0.0001$) (Table 1). In addition, zygotes centrifuged for 10 s were significantly heavier than those centrifuged for 3–4 s ($F_{6,36} = 2.39$, $P < 0.048$). Similar results were observed for the soaking experiments in which zygotes were heavier than the other two stages ($F_{2,18} = 7.75$, $P < 0.0016$). There were, however, no differences among soaking time treatments.

Centrifugation and soaking time experiments: P. calcar—The effects of centrifugation and soaking time were measured for only ova and gastrulae. The AFDWs of ova were significantly heavier than for gastrulae ($F_{1,12} = 10.29$, $P < 0.0075$) (Table 1). Ova and gastrulae soaked for 2 min were significantly heavier than those soaked for 30 s ($F_{2,12} = 4.48$, $P < 0.0352$) but equal to those soaked for 3–4 s.

Centrifugation and soaking time experiments: P. exigua—There were no significant differences in the AFDWs of any stages or treatments ($P > 0.05$).

Multiple rinses experiment—There were no significant differences in the AFDWs among the number of rinses or developmental stages of *P. regularis.* Visual estimates, however, indicated that there was a considerably larger proportion of ruptured ova and embryos with increased numbers

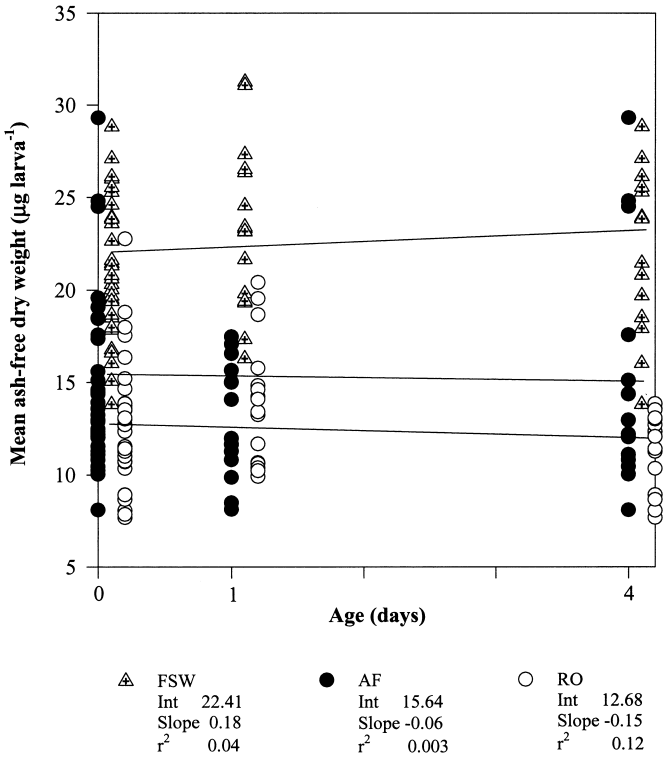

Fig. 1. Different rinsing solutions experiment. The mean AFDW of developmental stages of *P. calcar* rinsed with FSW, 3.4% AF, or RO. Developmental stages include zygotes—0 d, gastrulae—1 d, and early brachiolariae—4 d old. The FSW and RO symbols are slightly offset from the ages at which they were sampled to improve clarity.

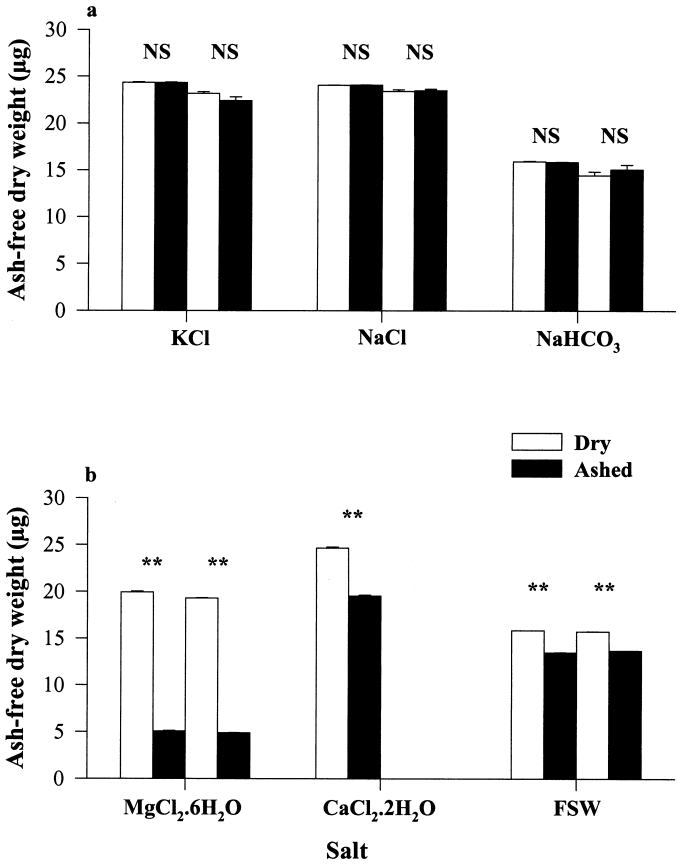

Fig. 2. Inorganic salts experiment. Various salt solutions were processed for the AFDW technique. Samples were weighed dry and ashed to determine weight changes due to this technique. (a) Salts without water of crystallization, and (b) salts with water of crystallization. **, significance to 0.0001; NS, not significant.

of rinses. Three rinses damaged up to 100% of the ova, zygotes, and gastrulae.

Effect of rinsing solutions—The AFDW of ova of *P. calcar* rinsed in FSW was double that of ova rinsed in RO or AF ($F_{8,45} = 2.50$, $P < 0.0246$) (Table 1). No differences in the AFDW of ova were found between RO and AF in three of five batches. For the other two batches, ova processed in RO were heavier than those treated in AF in one batch, while the other batch showed the opposite result. There was a strong treatment effect on gastrulae rinsed with FSW, which were heavier than those rinsed with either of the two other solutions ($F_{2,6} = 520.17$, $P < 0.0001$). Weights of gastrulae rinsed in RO or AF did not differ. Samples rinsed in FSW had a positive slope through development, while samples rinsed in AF or RO had negative slopes, but only the latter was significantly different from zero ($F_{1,88} = 11.60$, $P < 0.001$) (Fig. 1). An analysis of covariance with time and treatment as the covariates showed that the slope of FSW was significantly different from the slopes of AF and RO ($F_{1,2} = 25.54$, $P < 0.001$), which, in turn, were not significantly different from each other.

Effects of drying and ashing on the weight of inorganic salts—There were no differences between the dry and ashed weights of KCl, NaCl, and $NaHCO_3$. By contrast, salts with water of crystallization, $MgCl_2 \cdot 6H_2O$ and $CaCl_2 \cdot 2H_2O$, lost 75 and 20% of their weight after ashing, respectively (Fig. 2). The dry weights of these salts were significantly higher than the ashed weights ($P < 0.0001$). Similarly, FSW salts lost between 13.6 and 14.8% of their dry weight after ashing ($P < 0.0001$).

Handling and manipulation effects—Different preparatory methods of the AFDW technique influenced the organic weights of the ova, zygotes, and gastrulae of the three *Patiriella* species. Centrifugation affected the weight of zygotes of *P. regularis.* Regardless of time of centrifugation, zygotes were significantly heavier than ova or gastrulae. Other studies of echinoderm embryos report an increase in AFDW during pregastrula development (i.e., nonfeeding stages) and suggest that the gain in weight was due to uptake of dissolved organic matter (DOM) (Shilling and Manahan 1990; Shilling and Bosch 1994). The ova and zygotes of *Patiriella* spp., however, show no or minimal transport of dissolved free amino acids, DFAA, and presumably DOM (Moreno 1996), similar to the early developmental stages of other marine invertebrates (Epel 1972; Manahan 1983*a*,*b*). In this study, we purposefully processed ova and zygotes of *P. regularis* from the same batch to try and address the unusual weight increase observed from other studies. For *P. regu-*

laris, the apparent weight increase that occurred within the 10 min it took for fertilization to occur suggests that a handling artifact or ion flux, rather than DOM uptake, may be a more likely explanation.

The weight of developmental stages might be expected to drop as soaking time increases if rinsing with freshwater caused major damage to these stages. This was not the case, and in some instances, samples soaked for longer amounts of time were significantly heavier. This increase in weight may be attributed to a higher quantity of water diffusing into the cytoplasm and being potentially retained during drying but lost during ashing (*see below*). These results indicate that the rinsing period should be as brief as possible.

Rinsing solutions and inorganic salts—Comparison of the effect of the three different rinsing solutions revealed that the AFDW of developmental stages rinsed with FSW was twice that of samples rinsed with AF or RO, while there were no consistent differences between the AF and RO treatments. Heavier weights of samples rinsed in FSW could be attributed to several reasons: (1) rinsing with AF or RO caused massive and similar disruption and loss of organic materials from ova; (2) some inorganic salts present in FSW sublimate during the ashing process; and/or (3) some sea salts are hygroscopic and contain water, which remains after drying but is removed after ashing, thus resulting in overestimation of the AFDW.

Manipulation of soaking and centrifugation times as well as rinsing protocols revealed that even though physical damage was evident in specimens in some treatments (e.g., multiple rinses), there were few significant differences in weight among treatments. This suggests that unless leakage occurs within the 4–10 s it takes to drain a sample, then the rinsing procedure does not cause the sample to lose constituents. Weights of damaged ova were not quantitatively different from those that were not damaged, even in cases where extensive rupture occurred, as seen in the multiple rinse experiment.

Experiments with inorganic salts showed that some salts lost a considerable proportion of their weight after ashing. If ashing burns off water of crystallization, this problem is not restricted to the surface of the ova and embryos. Ions present intracellularly in all developmental stages have the potential to bind water, thus affecting the measurement of the AFDW. We suggest that water is retained by intracellular ions while the samples are drying at 80°C and is lost during ashing at 480°C. Large changes in the internal ionic composition of ova and larvae through development might have the potential to influence water retention and estimation of AFDW. This phenomenon may be of particular importance during and after fertilization, when a cascade of cellular ionic and biochemical processes takes place (Epel 1990), which may partially explain the increase in weight of zygotes observed for *P. regularis* and in other studies (Shilling and Manahan 1990; Shilling and Bosch 1994).

The AFDW technique—Significant differences in the AFDW of *Patiriella* ova, zygotes, and gastrulae resulted from sample manipulation and processing. This emphasizes that care must be taken when making inferences about weight changes among developmental stages of asteroids and other marine invertebrates because each species and stage may be affected differently by each processing step in the AFDW technique.

The three *Patiriella* species studied here exhibited different susceptibilities to handling and processing. The developmental stages of *P. exigua* were not affected by the AFDW procedures, perhaps due to the presence of a tough, highly modified fertilization envelope, a feature associated with evolution of benthic development (Cerra and Byrne 1995). In contrast, the developmental stages of *P. regularis* and *P. calcar* were affected by the various procedures of the AFDW technique. The fertilization envelopes of these species are less robust, typical of planktonic developers (Cerra et al. 1996), perhaps making them more susceptible to damage during procedures of the AFDW technique. The ova of *P. calcar* and *P. exigua* are of similar size, but their developmental stages exhibited striking differences in susceptibility to the AFDW procedures. This may be due to differences in ova lipid and protein composition between these two species (Byrne et. al 1999).

The magnitude of the weight losses found in the inorganic salts experiment is similar to the missing fraction reported for biochemical studies of larvae of marine invertebrates (Gnaiger and Bitterlich 1984; Jaeckle 1995), where ''incomplete or excessive recoveries, up to 15–20% of the ash-free dry weight,'' were a consistent error (Gnaiger and Bitterlich 1984). This has been suggested to indicate the presence of inherent errors in the AFDW technique (Gnaiger and Bitterlich 1984). Various studies have concluded that the remaining fraction or missing part is made up of components not sampled by the techniques that extract lipid, carbohydrate, and protein (Lucas and Crisp 1987; Jaeckle 1995). Although it is probable that some biochemical components such as amino acids are not accounted for by the techniques currently employed, the similarity between the missing fraction (≈20%) and the weight loss of FSW salts (≈14%) is intriguing. The weight loss associated with the AFDW technique potentially due to the loss in weight of inorganic salts warrants further investigation. Possible solutions to this problem include the use of higher temperatures or strong desiccants to dry the samples (e.g., phosphoric oxide, P_2O_5). The use of higher temperatures, however, may introduce additional problems through the loss of other compounds. As an alternative, samples could be lyophilized, weighed, and then ashed. Differences between the weights of samples that were dried or lyophilized would determine if lyophilization was an appropriate drying method. Unfortunately, other methods that directly measure organic content (e.g., bomb calorimetry) are standardized to the AFDW technique and cannot be used independently. Although the potential influence of water of crystallization on the AFDW technique is plausible, further experimentation is required to fully resolve this issue.

Studies that measure growth (as biomass changes) of marine invertebrate larvae remove sea salts from the larval surface prior to analysis (Dobberteen and Pechenik 1987; Jaeckle and Manahan 1989; Shilling and Manahan 1990; Hoegh-Guldberg 1994). The conclusion that inorganic salts are hygroscopic and hold water at 80°C demonstrates that

the removal of sea salts from the surface of ova and embryos is necessary. Care must be exercised during the processing of samples because it may result in unwanted effects. Because marine invertebrates are generally hyperosmotic to their environment (Pierce 1970; Oglesby 1981), their biomass is made up of a large quantity of inorganic ions. Hyperosmoticity and hygroscopicity together probably influence the weight of marine invertebrate larvae and may be of particular importance to those stages that experience marked ionic fluxes such as during fertilization. The increase in AFDW observed after fertilization may be due to the volume expansion associated with the formation of the fertilization envelope and the concurrent increase in inorganic ions and thus water of crystallization. The lower weight of ova, without a protective fertilization envelope, may be caused by damage and loss of contents during processing. Finally, the subsequent decrease in weight at the gastrula stage may be due to the loss of the fertilization envelope and associated structures and salts.

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Eighty years of spatially coherent Austrian lake surface temperatures and their relationship to regional air temperature and the North Atlantic Oscillation

Abstract—Eighty years of monthly mean lake surface temperature (LST) data from eight lakes in the northern perialpine area of Austria show a high degree of coherence among lakes in all seasons and reflect much of the temporal structure of the regional air temperature. Coherence is least in winter because of the distorting effect of varying periods of ice cover. In spring, regional coherence in meteorological driving forces that are essentially uncorrelated with air temperature (e.g., geostrophic wind speed) contribute to the coherence in LST, presumably by partially determining the timing of the onset of stratification. In summer, spatial coherence in LST appears to be related directly (via the radiation balance) and/or indirectly (via air temperature) to large-scale variations in high-altitude cloud cover. Correlations of the Austrian LSTs with (1) seasonal indices of the North Atlantic Oscillation (NAO), (2) the timing of spring ice break-up in Finland, and (3) air temperatures in northern and western Europe, suggest that from autumn to spring, spatial coherence of LST in central Europe is related to the dominance of the weather by large-scale climatic processes occurring over the North Atlantic, whereas in summer the processes responsible are more regional in nature. The influence of the NAO on LST is greatest in low-lying lakes in which periods of ice cover are infrequent and short.

Lake surface temperature (LST) is one of the most important physical parameters of any lacustrine system. On the one hand, it reflects meteorological forcing more immediately and more sensitively than any other lake parameter; on the other hand, it is strongly related to the mean temperature of the photosynthetically productive zone and thus plays a major role in lake biology. Because many cellular processes are temperature dependent, epilimnetic temperature conditions are important not only for individual pelagic and littoral organisms but for entire aquatic ecosystems (e.g., Regier et al. 1990; Arnell et al. 1996 and references therein). LST is comparatively easy to measure (traditionally several cm below the lake surface). Accordingly, existing time series of LST tend to extend further back in time than those of most other lake parameters and thus comprise a valuable source of data for studying the effects of climatic forcing on lakes on long timescales.

The five main heat-exchange processes that determine the heat balance of a lake depend essentially on only four meteorological variables; viz. cloud cover, water vapor pressure, wind speed, and air temperature (Edinger et al. 1968; Sweers 1976). Of these, air temperature is of greatest interest, in view of its importance in the current climate change debate (e.g., Nicholls et al. 1996). LST tends asymptotically toward an equilibrium temperature (Edinger et al. 1968) that can be close to the ambient air temperature (Arai 1981) but can also deviate strongly from this because of radiative heat exchange and wind mixing (Dingman 1972; Arai 1981). However, despite the apparent weakness of the direct causal connection, air and surface water temperature are often highly correlated on both short and long timescales (McCombie 1959; Shuter et al. 1983; Livingstone and Lotter 1998; Livingstone et al. 1999). Long-term comparisons of LST with surface air temperature are therefore of interest to determine to what extent general conclusions that have already been drawn with respect to the manifestations of global and regional climate change on air temperature (Nicholls et al. 1996) may also be applicable to LST.

Surface air temperatures in Europe are highly correlated over distances corresponding to synoptic-scale meteorological processes (~1,000 km), especially in winter and spring (Table 1). Taken in conjunction with the fact that the LSTs of individual lakes are usually highly correlated with air temperature locally (e.g., Livingstone and Lotter 1998), this suggests that LSTs may also be correlated over similarly large distances. Various studies have shown LSTs to be correlated on a much smaller scale—e.g., several tens of km within individual lake districts (Magnuson et al. 1990; Benson et al. 2000; George et al. 2000)—but work by Benson et al. (2000) suggests that the spatial scales involved may indeed be much greater than this. The question of the geographical extent of the correlation between LSTs is essentially one of whether the LST of a given lake can be profitably considered as the local manifestation of a spatially coherent synoptic-scale response to synoptic-scale meteorological processes, as opposed to an isolated local phenomenon. This question is of particular interest in view of the fact that air temperatures over large regions of the Northern Hemisphere, including most of Europe, appear to be determined to a large degree by climatological processes occurring over the North Atlan-