Addition of dissolved nitrogen and dissolved organic carbon from wild fish faeces and food around Mediterranean fish farms: Implications for waste-dispersal models

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Abstract

Leaching of ammonium (NH₄⁺) and dissolved organic carbon (DOC) from food pellets used at three fish farms in the Mediterranean Sea and the faeces of four different species of farm-associated wild fish (Trachurus mediterraneus, Mugil cephalus, Trachinotus ovatus and Boops boops) were determined. They were placed in seawater and agitated slowly (5 cm s⁻¹) to reflect natural conditions during their fall to the sediment. Two temperatures were tested, 25 °C and 15 °C, to assess the influence of seasons on leaching rates. Leaching from fish faeces was generally higher compared to food pellets. T. mediterraneus faeces leached more NH₄⁺ and DOC than M. cephalus, T. ovatus and B. boops. The results showed that there is an important addition of NH₄⁺ and DOC to the water column during sinking of the faeces and that this is species-dependent. Water turbulence and faeces composition seemed to have a higher influence than temperature on the leaching process. Due to the high abundance and biomass of farm-associated fish in the Mediterranean and their capacity to remove waste, they appear to be an important component for models that predict the impact of aquaculture. Large biomasses of wild fish at fish farms may reduce the impact on benthic systems but increase the nitrogen and carbon loads into the water column, affecting the pelagic system and modifying the spatial dispersion of wastes.

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Keywords: Ammonium; Aquaculture; Dissolved organic matter; Farm-waste dispersal; Leaching; Wild fish

1. Introduction

As sea-cage aquaculture continues expanding rapidly along the Mediterranean coast both in the number of farms and production (FAO, 2003), it is necessary to be more accurate when predicting potential impacts of this practice. Consequently, much research has focused on modelling the dispersal of sea-cage farm wastes to infer the impact of aquaculture more precisely and thus improve management actions and find the most suitable places to locate farms (see Cromey and Black, 2005 for review).

The environmental modifications that sea-cage aquaculture produces on the benthos (Karakassis et al., 2000) and water column (Pitta et al., 1999) are quite well
known. Moreover, some works have detected the effect of aggregated wild fish in reducing the impact on the benthos (e.g. Katz et al., 2002; Vita et al., 2004). There are models that include wild fish as removers of feed wastes (http://www.meramed.com) but they are only considered as a sink of organic matter. However, the associated ichthyofauna continues the process of transformation of wastes through excretion and defecation, projecting the nitrogen and carbon from the food pellets to the pelagic environment. However, even though it is possible to infer the quantity of waste material removed by wild fish, the effect of their defecation on the nutrient fluxes is more difficult to assess (Cromey and Black, 2005).

Leaching of nutrients from fish faeces during sinking has not been studied except for some cultivated species like salmon (Phillips et al., 1993; Tlusty et al., 2000; Chen et al., 2003), sea bass and sea bream (Magill et al., 2006). Due to the high abundance and biomass of farm-associated wild fish in the Mediterranean (Dempster et al., 2002, 2004) we hypothesized that they may have an important role by changing the pattern of addition of nutrients to the environment, reducing the impact on the benthos by feeding on the lost pellets and increasing nutrient levels in the water column. Also, we considered that water temperature should be an important factor that affects leaching rates. Therefore, we compared the NH$_4$ and DOC leaching from the faeces of four important species (Trachurus mediterraneus, Trachinotus ovatus, Boops boops and Mugil cephalus) to that of food pellets at two temperatures, 25 °C and 15 °C (summer and winter conditions respectively). Since the aggregation of these species of wild fish around Mediterranean fish farms seems to be a general phenomenon (Dempster et al., 2002, 2005; Smith et al., 2003; Thetmeyer et al., 2003), the data obtained here may help to develop more accurate models to predict the effect of aquaculture on coastal environments.

2. Materials and methods

Wild fish aggregated around three fish farms separated by 50 km along the south-east coast of Spain (Fig. 1) were captured. The farm at Campello was 3.2 km off the coast at an average depth of 28.6 m; the farm at Guardamar was 3.7 km from shore at a depth of 22.6 m; and the farm at Altea was 2.8 km offshore at an average depth of 34 m. Mean current speed at farms were measured at every site at 15 m depth. Current speeds were 5.2 cm/s at Campello (40 days of record), 3.5 cm/s at Guardamar (44 days of record) and 3.4 cm/s at Altea farm (51 days of record, Workhorse Sentinel Acoustic Doppler Current Meter). The maximum temperature in summer is 27 °C and 13 °C is the minimum temperature of water during winter season. All farms reared sea-bass (Dicentrarchus labrax) and sea-bream (Sparus aurata). Fish were caught during December 2004 and January 2005; the target species were the mugilid M. cephalus, the carangids T. mediterraneus and T. ovatus and the sparid B. boops. Due to the different abundance of the species around each farm, every species was captured in a single farm; M. cephalus was captured in Guardamar, T. ovatus in Altea, and T. mediterraneus and B. boops were fished in Campello farm. Food pellets used to feed cultivated fish were collected from the three

Fig. 1. Map of the three Sea Bream and Sea Bass farms on the SE coast of Spain where farm-associated wild fish were collected from.
farms and tested in the same way as faecal pellets. Upon arrival at laboratory, the last portion of the intestine was dissected and emptied. This method of collecting faeces could lead to an overestimation of the nutrients that the fish excretes because of a possible reabsorption in the distal gut. However, due to the lack of information about faeces composition in the species we tested, we followed the methodology of Chen et al. (1999), who showed that there were no significant differences in carbon and nitrogen content of the faecal material collected from any part of the last 4 cm or so of the distal hindgut of Atlantic salmon (Salmo salar L.).

Only fish with more than 1.2 g of faeces present were used for analysis. Faecal matter from three individuals from each species was used for the experiments. Faeces were divided in eight parts of approximately 0.15 g each, weighed and dropped in different 50 ml sample jars with filtered seawater (glass fiber filter of 0.45 μm).

Two experimental temperatures (25 °C and 15 °C) were used to reflect seasonal variances. Jars were agitated with an automatic shaker at 5 cm s\(^{-1}\) following Chen et al. (1999), for simulating turbulence during settling. Preliminary studies showed that the first minutes of immersion produced the maximum leaching rate so we analyzed samples at 1, 5 and 30 min. Also, we measured 60 min as the maximum possible settling time based on the slow sinking rate of faeces and maximum depths of the fish farms studied. Each sampling time was replicated twice.

Finally, water samples were analyzed for dissolved organic carbon with a SHIMADZU TOC-500A analyzer and ammonium was analysed with the Indophenol method (Grasshoff et al., 1999). Filtered seawater used in each experiment was analysed to determine initial DOC and NH\(_4^+\) concentrations present in samples. The obtained results have been fitted to the first-order kinetic model:

\[
y = a(1-e^{-kt})
\]

where \(y\) is the mass of DOC (mg of wet weight) or NH\(_4^+\) (μg of wet weight) per faeces gram and \(t\) is time, expressed in minutes; \(a\) and \(k\) are fit parameters; \(a\) represents the maximum leaching concentration under these conditions, expressed as leached mass per faeces gram; \(k\) is the first order kinetic constant (min\(^{-1}\)) and represents the velocity of the leaching process.

Differences in leaching rates were tested using the Chow-test (Kennedy, 1998, Troedsson et al., 2002), with the formula:

\[
F = \frac{\sum S^2_{\text{pool}} - \left(\sum S^2_{A} + \sum S^2_{B}\right)}{K} \frac{K\left(n_{A} + n_{B} - 2K\right)}{K\left(n_{A} + n_{B} - 2K\right)}
\]

This test compares the residual sum of squares for the pooled samples \(\sum S^2_{A}\) and \(\sum S^2_{B}\) that represent the residual sum of squares for the two regressions \(A\) and \(B\). \(K\) is the number of regression parameters, while \(n_{A}\) and \(n_{B}\) are the samples sizes of \(A\) and \(B\). If the \(F\)-value was greater than the tabulated \(F\)-distribution for \(p=0.05\) for \(K\) degrees of freedom in the numerator and \(n_{A} + n_{B} - 2K\) degrees of freedom in the denominator, then the hypothesis that the regression parameters are the same for both data sets was rejected.

### Table 1

\(a\), \(k\) and \(r^2\) values for NH\(_4^+\) and DOC leaching regressions for the 4 species of farm-associated wild fish plus food pellets at the two experimental temperatures (25 and 15 °C)

<table>
<thead>
<tr>
<th></th>
<th>25 °C</th>
<th>15 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a) (μg g(^{-1}))</td>
<td>(k) (min(^{-1}))</td>
</tr>
<tr>
<td>NH(_4^+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. cephalus</td>
<td>107.77</td>
<td>0.71</td>
</tr>
<tr>
<td>T. ovatus</td>
<td>284.50</td>
<td>0.14</td>
</tr>
<tr>
<td>T. mediterraneus</td>
<td>709.20</td>
<td>1.20</td>
</tr>
<tr>
<td>B. boops</td>
<td>476.90</td>
<td>0.40</td>
</tr>
<tr>
<td>Food pellets</td>
<td>169.70</td>
<td>0.20</td>
</tr>
<tr>
<td>DOC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. cephalus</td>
<td>5.55</td>
<td>0.17</td>
</tr>
<tr>
<td>T. ovatus</td>
<td>15.87</td>
<td>0.13</td>
</tr>
<tr>
<td>T. mediterraneus</td>
<td>29.76</td>
<td>0.15</td>
</tr>
<tr>
<td>B. boops</td>
<td>12.26</td>
<td>0.13</td>
</tr>
<tr>
<td>Food pellets</td>
<td>8.94</td>
<td>0.02</td>
</tr>
</tbody>
</table>

3. Results

Results showed that there were differences between the leaching rates of food pellets and the fish faeces
leaching, thus indicating that the presence of wild fish may strongly modify the nutrients cycle around sea cages. The Chow test showed that there were always significant differences in the leaching rates of all the pairwise comparisons at both temperatures. However, there was an exception for *M. cephalus* which showed no significant differences to food pellets at 25 °C and its DOC leaching rate again showed no differences at both 25 °C and 15 °C (for this non-significant results *F*-values ranged 1.44 to 3.85; *p*<0.05; critical value *F*(2,6)=5.14).

The pattern of ammonium and DOC leaching of faeces of the four species of farm-associated wild fish was statistically different among them. Further, as expected, leaching amounts differed greatly when wild fish faeces and food pellets were compared, with substantially greater amounts leached from the faeces of all species except *M. cephalus* (Table 1).

Faeces from different species of wild fish leached different quantities of ammonium to the seawater, with total leaching amounts higher than food pellets, except or *M. cephalus* (Fig. 2). *T. mediterraneus* was the species that leached the greatest amounts of NH$_4^+$ both at 25 °C and 15 °C (709.2 and 623.02 μg NH$_4^+$ g$^{-1}$, respectively), followed by *B. boops* (476.9 and 175.28 μg NH$_4^+$ g$^{-1}$) and *T. ovatus* (284.5 and 278.63 μg NH$_4^+$ g$^{-1}$). *M. cephalus* faeces, however, leached a similar total amount to the food pellets (107.7 and 70.6 μg NH$_4^+$ g$^{-1}$, Table 1).

For dissolved organic carbon, the pattern was similar to ammonium (Fig. 3). *T. mediterraneus*, again, leached the greatest amount; 29.7 and 33.75 mg DOC g$^{-1}$ at 25 and 15 °C. *T. ovatus*, followed by *B. boops*, had the next highest amounts of carbon leaching from faeces (Table 1). Also, *M. cephalus* leached of the least DOC (5.55 and 3.23 mg DOC g$^{-1}$ at 25 and 15 °C), even lower levels than those recorded for food pellets (8.94 and 4.84 mg DOC g$^{-1}$).

The leaching velocity (*k*) for NH$_4^+$ was always higher than that for DOC, showing that the addition of ammonium is generally faster at the initial stage when the faeces and food pellets first make contact with the sea water (Figs. 2 and 3). Food pellets always showed the lowest *k* values, except for *T. ovatus* for ammonium at 25 °C (Table 1). Surprisingly, the first-order kinetic constant was generally higher at 15 °C.

![Fig. 2. NH$_4^+$ leaching for the faeces of the four species and the food pellets. △:25 °C; ●:15 °C. Solid line represents the first-order kinetic fit obtained for 25 °C; dashed line represents the first-order kinetic regression fit for 15 °C.](image-url)
No consistent pattern in rates of leaching was evident when comparisons were made between the two tested temperatures although significant differences in leaching rates existed for all the species except for *T. mediterraneus* for NH$_4$+ and for *M. cephalus*, *B. boops* and food pellets for DOC (Table 2). Elevated temperatures did not cause a higher leaching rate; in some species, such as *T. mediterraneus* and *T. ovatus* for DOC, the rate at 15 °C was higher than at 25 °C (Figs. 2 and 3).

### 4. Discussion

Wild fishes that aggregate around fish farms and feed on the lost food pellets influence the environmental impact by excreting nitrogen and carbon to the water column, thereby reducing the input to the benthos. Our results demonstrate that there was an important input of NH$_4$+ and DOC from the faeces to the pelagic system, very fast in the first minutes. This therefore, reduces the quantity of organic matter that reaches the seafloor. Uneaten food pellets start leaching nutrients as soon as they contact water; however, they are rapidly eaten by wild fish in a high proportion (Sanchez-Jerez et al., unpublished data). Aggregated wild fish reduced the sedimentation of the total organic wastes at one Mediterranean farm by up to 80% (Vita et al., 2004). The wild fish component has thus far been inadequately incorporated into models of the dispersal of farm wastes. The effect of wild fish has been represented as a sink of organic matter, but wild fishes also redistribute a proportion of the nutrients by defecation, something almost ignored until now (Chen et al., 2003). This addition of

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**Table 2**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>NH$_4$+</th>
<th>DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. cephalus</em></td>
<td>11.97*</td>
<td>4.92</td>
</tr>
<tr>
<td><em>T. ovatus</em></td>
<td>7.57*</td>
<td>7.97*</td>
</tr>
<tr>
<td><em>T. mediterraneus</em></td>
<td>3.67</td>
<td>5.73*</td>
</tr>
<tr>
<td><em>B. boops</em></td>
<td>31.93*</td>
<td>3.29</td>
</tr>
<tr>
<td>Food pellets</td>
<td>9.41*</td>
<td>2.64</td>
</tr>
</tbody>
</table>

Tabulated $F$ value for $p=0.05$ is $F_{(2,6)}=5.14$.

Asterisk indicates significant differences.
nutrients and smaller particles to the water column should increase the dispersion coefficient and also provide a source of nutrients to the phytoplankton and DOC to bacteria, favouring a potential eutrophication process of the water column (Pillay, 2004). Hall et al. (1990), estimated a loss of dissolved carbon of 6 to 55% of total input to the farm. Faeces have a very high solubility potential (Thlusty et al., 2000) and experience a process of disaggregation during sinking. Consequently, there is a higher surface area of faeces in contact with the water and therefore it increases the contribution of DOC to the water column. The increment of DOC may modify the microbial loop and therefore, the structure of pelagic communities, and can be an easy tool for detecting the influence of this process on the pelagic system (Valiela, 1995).

All of the species we tested are abundant around many fish farms in the western part of the Mediterranean (Dempster et al., 2002) and species such as B. boops and mugilids are commonly observed as dominant components of the aggregated fish fauna in the eastern Mediterranean (Smith et al., 2003; Thetmeyer et al., 2003). Consequently, the addition of carbon and nitrogen through wild fish faeces to the pelagic environment is likely to be a general phenomenon around farms in the Mediterranean Sea. Moreover, these aggregations can reach an elevated biomass; up to 14.4 tonnes/10,000 m³ (Fernandez-Jover et al., unpublished data).

To properly model the influence of wild fish, it is important to have accurate estimates of the aggregated fish assemblage composition and biomass around farms because of the large differences we have demonstrated in the faeces leaching rate among species. For example, T. mediterraneus was the species that added the most nutrients into the water column. This species is present at many farms in south-east Spain and occurs throughout the year but with seasonal fluctuations in abundance (Fernandez-Jover et al., in press). The presence of this species in high abundance and biomass may be critical when modelling the dispersal of nutrients because of the rapid leaching rates of their faeces. Other carnivorous species, such as T. ovatus also had a high leaching rate. However, M. cephalus faeces leached nitrogen and carbon in a similar way as food pellets. The low leaching rate of feed may be related to its compact structure: the pellets remain stable over a longer period than faeces. Therefore, we would expect a significant change in the addition of DOC and NH₄⁺ to the water column would occur when species like the carnivorous T. mediterraneus or T. ovatus dominate the wild fish assemblages but that such changes would be lower when mugilids are the most abundant fishes.

The lower addition of DOC and NH₄⁺ of the non-carnivorous species such as mugilids could be due to the relatively longer intestine length of herbivores and detritivorous, as animals that feed on low-protein, high-roughage diets require longer guts in order to process typically poor quality food in the large amounts they require (Sibly, 1981). However, when omnivores and carnivores feed on the same type of food (food pellets), omnivores are likely to be more efficient in absorbing nutrients due to their longer intestines, and will therefore produce faeces of lower nutrient load. Moreover, gut length can vary with nutritional status, becoming shorter in herbivorous or detritivorous fishes when they are fed a higher-quality diet (Horn, 1989). If this occurs, aggregated fishes that set off on seasonal migrations after residing around a fish farm and having acquired a modified intestines adapted to a particular type of food (high energy food pellets), may not be able to fully exploit the nutrient content of a typical natural diet. Diets of wild T. mediterraneus aggregated at fish farms are known to contain predominantly lost feed and differ greatly from diets of their wild counterparts that typically feed on small juvenile fish and cephalopods in natural habitats (Fernandez-Jover et al., in press). Further research on this effect should determine if the feeding efficiency of fishes influenced by fish farms is diminished when they move away to more natural surrounds.

The sinking rate of faecal pellets is much slower and more variable than food pellets; therefore, they help to modify the pattern of nutrient addition to the environment. Preliminary experiments carried out up to 6 h (Fernandez-Jover et al., unpublished data) suggested that during the first hour, more than 90% of NH₄⁺ and DOC was leached to the water. Chen et al. (2003) found similar results to ours for leaching times; a rapid loss occurred in the first minutes after immersion. They considered determining leaching to 10 min as sufficient for modelling waste outflow from salmon farms. According to our results, 10–20 min is more suitable for adding the effects of wild fish to models, since that was the time range in which regressions became asymptotic. This could be due to the strong initial addition of NH₄⁺ and DOC which was present in the digested faeces and is liberated to the environment almost immediately, while the input of these compounds to the water column due to organic material metabolism is slower and more gradual. Sinking rates for the faeces of different species and at different levels of turbulence should also be determined for a more accurate evaluation of nitrogen and carbon input to the pelagic system.
The results we obtained for leaching rates at different temperatures provided no consistent pattern. We hypothesized that leaching rates would be higher at 25 °C. We detected significant differences for most of the comparisons but frequently the leaching rates at 15 °C were higher than at 25 °C. The explanation can be that faeces from different individuals were used to carry out the experiments at the two temperatures because of the small available quantity of faeces. Therefore, the water turbulence of 5 cm s\(^{-1}\), faeces composition (determined by diet and digestion) and compacting seemed to be more important factors determining the DOC and NH\(_4\)\(^+\) leaching rate. Leaching of NH\(_4\)\(^+\) and DOC from food pellets increased at 25 °C but was only significant for NH\(_4\)\(^+\). Since the composition of food pellets is relatively homogeneous compared to the composition of wild fish faeces, it is likely that the increment in NH\(_4\)\(^+\) leaching rate is, in this case, temperature-dependent. Higher temperatures generally increase metabolic processes. However, due to the short duration of our tests it may have had a limited effect on leaching processes. As a consequence, only leaching of compounds from faeces that have intestine-originated NH\(_4\)\(^+\) and DOC may occur over this short period. Preliminary experiments carried out for 6 h showed that temperature, in the same way, did not significantly increase the contribution of NH\(_4\)\(^+\) and DOC to the seawater. Therefore, the effect of temperature on the metabolic processes of evacuated faeces may only be detectable over even greater periods of time. Also, it should be taken into account that although leaching rates of the food pellets used at the three fish farms were very similar, differences in food compounds may influence the faeces composition and therefore the leaching rates. Further, due to the high number of fish without faeces in the distal gut, the sample size was limited to three fish per species which may have affected the accuracy of the results.

The most severe impacts of aquaculture are confined to within 50 m of the fish cages (Hansen et al., 1991; Holmer, 1991; Karakassis et al., 1999). Islam (2005) reviewed research regarding nitrogen and phosphorus budgets in coastal and marine cage aquaculture environments, and pointed out that an important part of the lost feed pellets and faeces are eaten by wild fish in the Mediterranean and they therefore reduce the impact of these two nutrients on the benthos. Further, daily or seasonal migrations of fish may help in transporting farm wastes to a wider area rather than the immediate vicinity of a fish farm. Through the use of stable isotopes, transfer of farm-originated carbon and nitrogen to the pelagic system and dispersion over scale of 1000 m has been documented; this suggests wild fishes and benthic resuspension are key elements that influence the spread of waste materials from fish farms (Sarà et al., 2004). Moreover, it has been proposed to ban fishing farm-aggregated fish to completely harness their capacity to reduce the benthic impact (Dempster et al., 2005).

Following the conceptual model for nutrient mass budget of Islam (2005), we can estimate the influence of wild fish on the total amount of nitrogen lost to the environment. Using a Food Conversion Ratio of 1.79 for sea bream (Lupatsch and Kissil, 1998), without the influence of wild fish, 22.6 kg of nitrogen would sediment (at 25 °C) for 1 ton of sea bream production, including both feed and faeces nitrogen. Therefore, there is a severe impact on the surrounding benthic communities. However, if we consider a scenario where wild fish consumed 80% of lost food pellets (Vita et al., 2004) and applying our results on faeces leaching rates (assuming that all the aggregation is composed of T. mediterraneus and applying its maximum faeces leaching rate at a water temperature of 25 °C), only 0.28 kg of nitrogen will reach the sea. This load of nutrients could be dispersed or assimilated by the pelagic communities and therefore reduce the impact on the benthic environment around fish farms.

Future models for dispersion of farm wastes should include losses by leaching to avoid any overestimation of nutrient input into sediments and ignore the addition of dissolved nutrients into the water column (Chen et al., 2003). Moreover, in marine fish, 50–70% of the nitrogen excreted occurs across the gills (Sayer and Davenport, 1987). The excretion of ammonium through this path is more important than that of the faeces, so this should be added to the input of NH\(_4\)\(^+\) from cultivated and wild fish to the environment. As wild fish reach a high quantity and biomass around Mediterranean fish farms, we also conclude that cultivated-fish faeces (e.g. Magill et al., 2006) and farm-associated wild fish faeces should be considered when modelling the dispersal of fish farm wastes for better understanding the impact of fish farms. Further research on food digestibility of farm-associated species, their daily and seasonal movements in the vicinity of farms to determine how much organic matter they may export from the vicinity of farms and in situ settling velocities of their faeces is necessary.

5. Conclusion

Food pellets used to feed the caged fish and the faeces of different species of aggregated wild fish leach DOC and NH\(_4\)\(^+\) at different rates throughout their sinking phase. Leaching from faeces was higher than from food pellets except for M. cephalus. T. ovatus and T. mediterraneus had the greatest leaching rates of DOC, three to 10 times higher than M. cephalus. On the other hand, T. mediterraneus showed NH\(_4\)\(^+\) leaching rates seven to nine times higher than...
M. cephalus and two times higher than the other species. Therefore, the relative environmental effect of sedimenting food pellets compared to the effect of sedimenting fish faeces may differ depending on the fish assemblage structure present at any given fish farm. However, water turbulence and faeces composition seemed to have a higher influence than temperature on the leaching process. Wild fish remove an important proportion of the solid wastes, thereby diminishing the impact on the seafloor and, at the same time, add nitrogen and carbon into the pelagic environment. Due to the high abundance and biomass of wild fish around Mediterranean fish farms, their role in influencing the benthos has been ignored or improperly reflected when modelling fish farm nutrient fluxes, probably resulting in overestimates of the impact on the benthos and underestimates of the dissolved proportion of wastes.

Acknowledgments

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