INTRODUCTION

The environmental impacts of aquaculture activities have drawn considerable attention over recent years (Subasinghe et al. 2009). To control the environmental impacts of aquaculture, some countries have instituted various limitations to either stocking density and feed use or concentrations of suspended solids, organic matter, and nutrients in effluents (Tacon & Forster 2003). Although environmental impact assessments are mostly based on the concentrations of particular compounds in the effluents of land-based farms, the pollution of receiving water bodies is mainly related to total waste loads per unit time (Rodrigues 1995). To estimate total waste loads many efforts have been made to date (e.g. Cho et al. 1991, Kelly et al. 1996, Aubin et al. 2011). The main purpose of load estimations is to predict the existing and future discharge of suspended solids, organic compounds, and nutrients, thereby allowing authorities to quantify the environmental impacts of activities with the greatest accuracy (Frier et al. 1995).

Waste loads from land-based aquaculture have commonly been estimated using mass-balance models containing 2 main approaches: hydrological and nutritional methods, which are also called chemical/limnological and biological/bioenergy methods, respectively. The hydrological method is based on the measurement of selected indicators between the inlet and the outlet of fish farms by taking the flow...
rate into consideration (Roque d’Orbcastel et al. 2008, Aubin et al. 2011). However, there may be uncertainties arising from changing farm practices and sampling methodologies, which can result in temporal variations in the suspended solids and nutrient concentrations in the effluent (Cho et al. 1991, Papatriphnon et al. 2005). This method may be required for frequent or continuous monitoring of inflow and outflow water quality to assure data precision and accuracy (Kelly et al. 1996).

The nutritional method has been developed as a simple and economical alternative to the hydrological method, and uses a simple nutrient balance and bioenergetics approach (Cho & Bureau 2001). The principle is based on the assessment of the difference between the nutrients and digestible energy supplied to fish and their body nutrient and energy gains. The proportion not retained by the fish for growth is released into the water and constitutes the waste emissions of the fish farm (Cho & Bureau 2001, Aubin et al. 2011).

Aquaculture is one of the fastest-growing industries in Turkey, having enlarged in volume by >20% between 2000 and 2010. Rainbow trout *Oncorhynchus mykiss*, with a 78 000 t yr⁻¹ production rate in 2010, was the dominant species, representing 99% of the production in Turkish inland waters (TURKSTAT 2012). Turkey is currently the third-largest farmed-fish producer in Europe and the top producer of rainbow trout (Deniz 2010). However, there are not only limited data on the estimation of the carrying capacity of Turkish river basins for land-based aquaculture, but also on the waste load estimation from trout farms (but see Pulatsü et al. 2004, Kırkağaç et al. 2009, Taşeli 2009, Tekinay et al. 2009, Bilgrin Yıldırım & Pulatsü 2011).

In the present study, we aimed to characterize the effluents of flow-through farms with different production capacities in a major rainbow trout site and estimate emissions of suspended solids, nitrogen, and phosphorus by the hydrological and the nutritional methods based on monthly data of water quality monitoring and annual average farm records. We also aimed to predict total nutrient flux from the farms into the Mediterranean Sea through the receiving stream using a simplified mass-loading model.

**MATERIALS AND METHODS**

**Study site and trout farms**

Eşen Stream, arising 2000 m above sea level and discharging into the Mediterranean Sea is the most significant rainbow trout *Oncorhynchus mykiss* production site of the flow-through raceways in Turkey. Its catchment comprises 50 farms, with a licensed capacity of about 7500 t yr⁻¹ and 176 million fry yr⁻¹. Most of the production takes place at the Çaygözü site, midstream in the basin. At this site, the 9 single-pass flow-through farms, with a total capacity of 4400 t yr⁻¹, are located along a stream reach of 2 km (Fig. 1).

The stream flow is diverted to a hydroelectric power generation in the upper stream region. A hydroelectric power plant (HEPP 1) is operated using the tail water of an upstream dam, and its outlet is discharged to the Çaygözü site. Then the flow is dammed up with a control gate (Regulator 1) below HEPP 1 and diverted into a conveyance canal. The conveyance canal water is used for irrigation (Regulator 2), and then, through a second hydroelectric power plant (HEPP 2), is finally discharged into Fethiye Bay part of the Mediterranean Sea (Fig. 1).

The total annual flow of the stream reach taking the discharges of all the trout farms was 373 × 10⁶ m³ yr⁻¹ over the study period, between March 2008 and February 2009. A significant portion of the total flow (312 × 10⁶ m³ yr⁻¹) was diverted into the conveyance canal by Regulator 1. About a third of this portion...
We monitored effluents of 3 farms with different production rates at the Çaygözü site. Farms I and II took their inflows from the stream receiving effluents from 4 other farms further upstream, with a total annual capacity of about 350 t yr⁻¹. Together with another one, these farms had a total annual capacity of 1200 t yr⁻¹, and their effluents discharged to the stream above the inflow of Farm III, in addition to the outflow from HEPP 1 (Fig. 1).

Annual production capacities of the monitored farms were 250, 750, and 2500 t yr⁻¹, respectively. Feed was distributed by hand twice a day at predetermined levels, changing according to size and biomass. Inflow rates were 1.1, 2.4, and 5.0 m³ s⁻¹ in ascending order by farm size, while the measured temperatures were 13.8 ± 0.7°C, 14.1 ± 0.7°C, and 13.1 ± 1.5°C during the study period, respectively. Although Farms II and III had settling ponds for solids removal, their overflow rates were high (>400 m³ m⁻² d⁻¹) compared with the recommended values for optimal effluent settling (Stewart et al. 2006). Farm I had a microscreen drum filter unit for effluent treatment, which was not active during the study period. It can be seen from the data gathered that none of the farms in the studied area employed effective effluent treatment practices.

Water sampling and analyses

Monthly water samples were collected from the inlets and outlets of the farms. Flow rates in the farms, stream and conveyance canal reaches were measured monthly by a digital meter (Hydro-Bios, Model RHCM). Suspended solids, chemical/biochemical oxygen demand, ammonia, and nitrite were analyzed on the sampling day, while the other analyses were completed on the following day.

At the inlet and outlet of each farm, water was characterized in situ by means of a probe with polarographic and thermistor type sensors (Yellow Spring Instruments, Model 55) for temperature, dissolved oxygen (DO), and oxygen saturation (SAT). Suspended solids (TSS) were determined by filtration using a glass fiber filter. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were determined via the 5 d incubation and open reflux methods, respectively. Total ammonia nitrogen (TAN), nitrite nitrogen (NO₂⁻N), and nitrate nitrogen (NO₃⁻N) were determined by phenate, colorimetric, and cadmium reduction methods, respectively. Soluble reactive phosphorus (SRP) and total inorganic phosphorus (TIP) were determined using the ascorbic acid method from filtered samples and hydrolyzed unfiltered samples, respectively. Total nitrogen (TN) was determined as nitrate following alkaline persulfate oxidation of unfiltered samples, while total phosphorus (TP) was determined as SRP following acidic persulfate oxidation. All laboratory analyses were performed according to standard methods (APHA 1998). Nutrient forms were determined using a spectrophotometer (Thermo, Model Helios-α).

Dissolved inorganic nitrogen (DIN = TAN + NO₂⁻N + NO₃⁻N), total organic nitrogen (TON = TN − DIN), and total organic phosphorus (TOP = TP − TIP) were calculated from total and inorganic fractions (APHA 1998).

Calculations and statistics

Differences (ΔC = Couts − Cin) between outflow and inflow concentrations and relative concentration differences (%ΔC = ΔC/Cin × 100) in the monitored farms were calculated for each parameter (Sindilariu et al. 2009).

The nutritional method was based on data provided by feed manufacturers, farm records, and literature (Papatryphon et al. 2005, Roque d’Orbcastel et al. 2008) (Table 1). Although there could be seasonal variations in farm parameters such as fish stocks and feeding rates, we used the average annual feed conversion based on interviews with the farmers to estimate the average daily feeding rate. The hydrological method was based on concentration differences (ΔC) of parameters and flow rate measurements (Roque d’Orbcastel et al. 2008, Aubin et al. 2011).

Normality of data was tested for each parameter using the Shapiro-Wilk test. The differences of each parameter from zero with normal and non-normal distribution were tested using a t-test and Wilcoxon test, respectively. Comparisons of normally distributed ΔCs by production rate (l s⁻¹ t⁻¹ of fish produced) were made using analysis of variance (ANOVA), whereas the signed-rank test (Wilcoxon), followed by the Student’s t-test, was used for non-normally distributed ΔCs. To understand the relations between farm production rates and the observed parameters, correlation coefficients were determined.

Nutrient flux (loading) into the Mediterranean Sea from the 9 land-based trout farms at the studied site was estimated by a simple mass-balance equation.
Mass flux in a given period can be calculated at a steady-state considering hydraulic balance (James 1993, Cox 2003). We estimated the mass-flux equation following the method of James (1993), with a slight modification. Because there is no inflow to the open channel system between the receiving stream reach and the sea (Fig. 1), we calculated the mass loading from the estimated annual average nutrient concentrations in the receiving stream reach and data on the total annual discharges in the conveyance channel. We neglected the other nutrient sources to estimate the specific loading from aquaculture activities and assumed the modeled nutrients as conservative. Accordingly, average annual concentration, discharge, and mass-balance were:

\[ C = \frac{L}{Q_1} \]  
\[ L = L_1 P_1 \]  
\[ L_{1,2,3} = \frac{Q_{1,2,3}}{C} \]  
\[ L_{1,2,3} = Q_{1,2,3} \left( \frac{P_1}{Q_1} \right) \]

where \( L \) is the total annual loading into the receiving stream reach from fish production (kg yr\(^{-1}\)), \( L_1 \) is the estimated waste loads per fish mass (kg t\(^{-1}\) of fish produced), \( P_1 \) is the total annual fish production (t yr\(^{-1}\)), \( C \) is the average annual concentration of TSS, TN and TP (kg m\(^{-3}\)), \( L_{1,2,3} \) are the total annual mass fluxes in the receiving stream (\( L_1 \)) reach and conveyance channel after Regulators 1 (\( L_2 \)) and 2 (\( L_3 \)), respectively (kg yr\(^{-1}\)), \( Q_{1,2,3} \) are the total annual flow rates in the receiving stream reach and conveyance channel after Regulators 1 and 2, respectively (m\(^3\) yr\(^{-1}\)).

### RESULTS

#### Effluent characteristics

The concentrations of the monitored parameters in the inflows and outflow of Farms I to III are presented in Table 2. The production rates of the farms had significant impacts on the concentrations of the monitored parameters in the effluents. The \( \Delta C \)s for most of the parameters were significantly different in the Farm III effluent compared with effluents of the other farms, except that the \( \Delta C \) for TAN was different from Farm I only. The \( \Delta C \)s for TSS and COD were comparable among the farms (Table 3).

The correlations between the \( \Delta C \)s and the annual production rates were very strong for DO and SAT (\( p < 0.001; r^2 = 0.88 \) and \( r^2 = 0.85 \), respectively), whereas there were significant but weaker correlations (\( p < 0.05; r^2 = 0.15 \) to 0.60) for BOD, COD, and the nutrient fractions.

The effect of trout culture on effluent water quality was manifested by a significant decrease in DO and SAT, and an increase (\( p < 0.05 \)) in suspended solids, BOD, COD, and nutrient concentrations compared with the inflow (Fig. 2). The mean decreases in DO and SAT were 24 and 23%, respectively, while the increases in the other parameters ranged between 8 and 65%.

The mean ratios of effluent TAN in DIN and TN were within the ranges of 60 to 75% and 24 to 44%, respectively. The ratios of NO\(_2-\)N/TN and TON/TN were 10 to 17% and 21 to 26%, respectively, with NO\(_2-\)N constituting only a little part. The effluent SRP within TIP ranged between 42 and 84%, whereas TOP represented most of the TP, with ratios between 66 and 78% (Table 4).

### Waste loads

The concentration of nitrogen and phosphorus fractions in the effluents displayed differences among the monitored farms. Therefore, the estima-
tions of emissions using the nutritional and hydrolog-
ical methods showed some variability (Table 5). De-
spite a high, negative bias at Farm I, the predicted
and measured TSS concentrations in the effluents
were highly similar. The measured TN concentra-
tions were in the range from 297 to 857 µg l⁻¹ at the
farms, whereas the predicted values were between
319 and 703 µg l⁻¹. TP concentrations by prediction
and measurement in the effluents of Farms I and II
were close, which was not the case at Farm III. How-
ever, overall predictions were well correlated with
measurements for all 3 parameters (Fig. 3).

Aside from an overestimation of measured TP for
Farm III, the load estimations based on the nutri-
tional and hydrological methods for suspended
solids and nutrients from trout culture activities
were almost the same for the 3 farms. Estimated
TSS loads by both methods overlapped at 278 kg
t⁻¹ of fish produced. An estimation of TN load of
44.3 kg t⁻¹ of fish by the nutritional method was
slightly higher than that by the hydrological
method. The nutritional and hydrological methods
estimated TP loads as 8.4 and 8.8 kg t⁻¹ of fish
produced, respectively (Table 6).

More precise estimations of nutrient loads also
reflected annual loading values. Estimates of annual
TN fluxes into the stream were 230 and 233 t yr⁻¹
and into the Mediterranean Sea 125 and 127 t yr⁻¹,
as assessed by the hydrological and nutritional meth-
ods, respectively. Estimates of annual TP fluxes were
46 and 44 t yr⁻¹ into the stream and 25 and 24 t yr⁻¹
into the Mediterranean Sea, respectively (Table 7).

Table 3. Changes (mean ± SD) in parameter concentra-
tions between inflow and outflow of 3 rainbow trout
Oncorhynchus mykiss farms. Values within the same rows not sharing a
common superscript letter were significantly different (p < 0.05,
Student’s t-test). Abbreviations as in Table 2
DISCUSSION

Effluent characteristics

Determined ranges and mean nutrient increases in effluents in our study are broadly consistent with the summarized data for several rainbow trout *Oncorhynchus mykiss* farms (Stewart et al. 2006, Sindilariu 2007, Aubin et al. 2009, Sindilariu et al. 2009, Tello et al. 2010). Effluent characteristics are also in concordance with the results of previous research on flow-through rainbow trout farms in the same region as our study (Tekinay et al. 2009, Bilgin Yıldırım & Pulatsü 2011).

It is well known that the nutrient concentrations in trout farm effluents are highly variable (e.g. Sindilariu 2007) and the effluent water quality is highly affected by farm management practices such as stocked fish size, stocking density, feed quality, feeding techniques, frequency of cleaning, etc., as well as temporal variations such as influent water quality and flow rate (e.g. Axler et al. 1997). Ammonia nitrogen can form 53 to 69% of total nitrogen wastes in the effluent of rainbow trout farms (Kajimura et al. 2004), but the ratio may increase up to 79% in some instances (Dalsgaard & Pedersen 2011). The ratios of TAN/TN in effluents in our study were unexpectedly lower than the literature values, suggesting that nitrification of ammonia and temporal variations in the samplings most likely played a significant role, as reported previously (Papatrephyon et al. 2005, Dalsgaard & Pedersen 2011).

The relatively high NO$_3$-N/TN ratios observed further support the impact of nitrification. High standard deviations in TAN concentrations in the present investigation could primarily be due to farm management practices and changes of
sampling time during the day, as suggested by Papa-
tryphon et al. (2005), Roque d’Orbcastel et al. (2008),
and Aubin et al. (2011). Although urea, amino acids,
and nitrogen excretion via the gills and/or skin and
mucus may comprise a considerable amount of the
soluble fraction of organic nitrogen (Kajimura et al.
2004), both soluble and particulate fractions may reach
up to 36% of TN (Foy & Rosell 1991). Because we did
not determine these fractions separately, our TON
values are indirectly consistent with the range of the

In contrast to earlier findings reporting that 60% of
TP loading was in the form of SRP (Foy & Rosell
1991), our findings are closer to the data of Roque
d’Orbcastel et al. (2008), who found a 31.2% SRP of
TP in trout farm effluent. The TOP/TP ratios were
between 66 and 78%, indicating that the majority of
phosphorus wastes in the monitored farm effluents
was in the organic fraction, presumably in the partic-
culate fraction organically bound in fecal and uncon-
sumed feed materials.

Waste loads

There were strong correlations between predicted
and measured concentrations of TSS, TN, and TP in
the effluents of the monitored farms. Our predicted
TN and TP loads were within the range of those pre-
sented by Bureau et al. (2003) and Roque d’Orbcastel
et al. (2008), who recorded 40.8 to 71 kg N and 7.5 to
15.2 kg P t⁻¹ of fish produced. Our predicted TSS
loads were in close agreement with those reported
by Bureau et al. (2003), who found 240 to 318 kg TSS
t⁻¹ of fish produced for land-based salmonid farms,
but higher than those by Roque d’Orbcastel et al.
(2008), who reported a load of 147.5 kg TSS t⁻¹ of fish
produced for rainbow trout. The inconsistency of TSS
prediction with the latter study could be due to differ-

| Load (kg t⁻¹ of fish produced) |
|-------------------------------|---|---|
| TSS                          | TN | TP |
| Predicted                    | 278 | 44.3 | 8.4 |
| Measured                     | 278 | 43.9 | 8.8 |

Table 6. Mean estimates of suspended solids and nutrient loads. Predicted: based on nutritional method; Measured: based on hydrological method. Abbreviations as in Fig. 3

| L₁: mass flux into the receiving stream reach from aquaculture activities; L₂: mass flux into the conveyance channel by Regulator 1; L₃: mass flux into Fethiye Bay after Regulator 2; TN: total nitrogen; TP: total phosphorus |
|--------------------------------------------------|---|---|
| Nutrient flux (t yr⁻¹)                            |
| TN      | TP | TN | TP | TN | TP |
| Predicted | 233 | 44 | 195 | 37 | 127 | 24 |
| Measured | 230 | 46 | 193 | 39 | 125 | 25 |

Table 7. Estimations of annual nutrient flux (t yr⁻¹) into the Esen Stream at the Çaygözü site and Mediterranean Sea using a simplified mass-flux equation. Predicted: loading data (L) for mass-flux calculation based on load value from the nutritional mass-balance method; Measured: L for mass-flux calculation based on load value from the hydrological mass-balance method. L₁: mass flux into the receiving stream reach from aquaculture activities; L₂: mass flux into the conveyance channel by Regulator 1; L₃: mass flux into Fethiye Bay after Regulator 2; TN: total nitrogen; TP: total phosphorus

Fig. 3. Relationships between measured (mean annual) and predicted concentrations of (a) total suspended solids (TSS), (b) total nitrogen (TN), and (c) total phosphorus (TP) in effluents of the 3 rainbow trout Oncorhynchus mykiss farms studied. y: predicted concentrations; x: measured concentrations
ences in the feed conversion ratio (FCR; 1.1 versus 0.85) and the assumed nutrient digestibility coefficients. Indeed, an improvement in the FCR could result in huge decreases in waste loads, as observed by Bilgin Yildirim & Pulatsü (2011).

There are many sources of uncertainties associated with the hydrological and nutritional methods. The primary uncertainties originate from the sampling process, especially its location in time and space for the hydrological method and its input data for nutrient-balance modeling (Aubin et al. 2011). Because of the above-mentioned temporal variations in solids transport and farm management, estimating quantitative waste outputs by hydrological or nutritional methods may lead to erroneous loading rates (Papatryphon et al. 2005, Sindilariu 2007, Roque d’Orbcastel et al. 2008). However, Papatryphon et al. (2005) suggested that, considering the nature of the nutrient emissions, the potential measurement error, and the variability associated with the environment and the farms, the differences between predictions and measurements may not seem important.

Therefore, despite the uncertainties, nutritional mass-balance modeling as a cost-efficient solution to estimate the release of waste can provide both fish farmers and authorities with valuable information on the environmental impacts of aquaculture farms, both active or soon to be activated (Aubin et al. 2011). Papatryphon et al. (2005) also suggested that nutritional mass-balance modeling should be the preferred method of environmental impact assessments for predicting nutrient emissions in various forms. Our study showed that a nutritional mass-balance method based on very simple inputs that are easily accessible, such as average annual feed use and fish production, as well as feed specifications, is capable of providing reliable estimations for suspended solids and nutrient loads, without seasonal data. Doubtless to say, an integration of more frequent observations in feed use and farm management practices will further increase the precision of the method. Yet the simple approach outlined in the present study can still help authorities during basin-scale planning of production for land-based operations.

High river-borne organic matter and nutrient inputs have been recognized as important sources of coastal eutrophication (Mallin et al. 1993, Rahm et al. 1996). This is particularly significant for an oligotrophic system like the Mediterranean Sea. Along the eastern Mediterranean coast, diffuse discharges from intensive cultivation practices and point discharges from urban waste water are the most significant sources of organic matter and nutrients carried to the sea by rivers and streams (Ludwig et al. 2009). Karakassis et al. (2005) calculated the contribution of a 100 000 t yr⁻¹ cage-aquaculture production to the total annual anthropogenic TN and TP loadings into the eastern Mediterranean as <8% using a mass-balance method similar to ours. This volume of production generated 12 × 10³ t N yr⁻¹ and 2 × 10³ t P yr⁻¹ in annual loadings or 120 and 20 kg t⁻¹ of fish produced, respectively. But the loads into the eastern Mediterranean from flow-through trout fish farming at the studied site were 28.4 to 28.9 kg N and 5.5 to 5.7 kg P t⁻¹ of fish produced. Although our estimations on TN and TP loads are almost a quarter of the estimations for marine cage farms by Karakassis et al. (2005), the results of the present study show that land-based trout farms may be considered significant aquacultural sources of nutrient flux into the coastal ecosystem.

CONCLUSIONS

The results of the present study showed that farm effluents have decreased DO and SAT values and increased TSS, BOD, COD, and nutrients compared with farm inflows. Estimations of the nutritional and hydrological mass-balance methods were well correlated with each other. The nutritional mass-balance modeling for capacity planning and basin-scale management of land-based aquaculture at a stage of environmental impact assessment seems to be a useful decision tool because of its cost efficiency and simple applicability. It was also possible to predict nutrient loading into the ultimate receiving coastal ecosystem using a simplified mass-flux model. Future studies and efforts should be focused on the determination of nutrient discharges from a variety of sources, together with aquaculture contribution to prepare a coastal zone management plan.

Acknowledgements. This study was supported by grants from The Scientific and Technical Research Council of Turkey (TUBITAK, Project No. 107Y084). We thank the fish farmers who collaborated with us on this study. The authors also thank Ramazan Uysal, Faruk Pak, Gazi Uysal, Ö. Aybike Topçuoğlu, and Gül Tunç Karaağaç for their invaluable help with data collection and laboratory analysis, and Prof. Dr. Ayşe Muhammetoğlu, Assoc. Prof. Yılmaz Emre and Prof. Dr. Ahmet Alp for his suggestions on this article.

LITERATURE CITED


Stewart NT, Boardman GD, Helfrich LA (2006) Treatment of rainbow trout (Oncorhynchus mykiss) raceway effluent using baffled sedimentation and artificial substrates. Aquacul Eng 35:166–178


Submitted: August 1, 2012, Accepted: January 30, 2013
Proofs received from author(s): March 23, 2013

Editorial responsibility: Tim Dempster, Trondheim, Norway