Acclimation guideline for *Litopenaeus vannamei* post-larvae

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The main parameters that need to be controlled when receiving and acclimating post-larvae are temperature, salinity, oxygen, and pH value. More parameters should be controlled to establish standardized acclimation protocols, as total ammonia concentration (TAN), alkalinity, CO_2 concentration. The total ammonia concentration is important to calculate the NH_3-N concentration, the undissociated form of ammonia also known as free ammonia nitrogen (FAN), causing intoxication at elevated levels. The CO_2 concentration can be measured or also be calculated by using the values for pH, alkalinity, salinity, and temperature. Table 6 and Table 7 are showing the relation for a typical range before and during acclimation.

Table 1 is presenting an overview of different hatcheries delivering post-larvae to Förde Garnelen since 2018. Shown are the mean values of water parameter at bag arrival for maximum the last 5 shipments.

Table 1 – Mean water parameter at bag arrival from different hatcheries delivering post-larvae to Förde Garnelen.

Hatchery	Origin	Distance to farm		n	Temp.	pH	Salinity	Oxygen	TAN	NH3-N	kH	CO2
		km	h		°C		g/L	%	mg/L	mg/L	°dH	mg/L
Suburban Seafood	GER Saxonia	541	6	3	23.2	7.0	30.9	202,0	1.8	0.01	6.5	16.7
Global Blue Technol.	US Texas	9.000	33	5	17.7	7.0	28.9	>265*	2.6	0.01	8.4	21.2
Amercian Penaeid	US Florida	9.000	33	5	17.7	7.0	31.0	>265*	8.6	0.03	7.4	18.6
Shrimp Improvement	US Florida	9.000	33	5	19.1	7.1	33.2	>265*	5.8	0.03	8.7	19.2
Molokai Seafarms	US Hawai	19.000	44	1	22.6	6.7	32.4	>265*	0.9	0.00	9.0	38.0

**above range of the measuring device*

<u>Temperature:</u>

Packing water is usually chilled to 19-23°C to reduce shrimp metabolism to maintain water quality at reasonable level. Table 1 shows arrival temperatures between 17-23°C for the air shipping. Changes are related to packaging, transport time and ambient temperature. Figure 1 clearly indicates also the high seasonal relevance.

<u>Salinity:</u>

Salinity of packing water is usually set to 29-33 g/L. Salinity is not changing during transport.

<u>pH:</u>

pH of packing water is usually set to 7.7-7.9. Usually no additional buffer (as for example TRIS buffer), except the natural carbon buffer system of seawater, are used. Table 1 shows arrival pH between 6.7-7.1. The reduction is caused by the excretion of CO_2 and the accumulation in the water as the sealed plastic bags interrupt the gas transfer into the atmosphere. Usually the effect is minimized by having a large void (gas space filled with pure oxygen) above the water.

Additionally, the carbon buffer system in the packing water may also help to stabilize the pH value by adsorbing a part of the CO_2. The drop in pH value can be increased by elevated water temperatures, longer transport times or too high stocking densities during transport resulting in a higher metabolic rate of the animals to excrete higher amounts of CO_2.

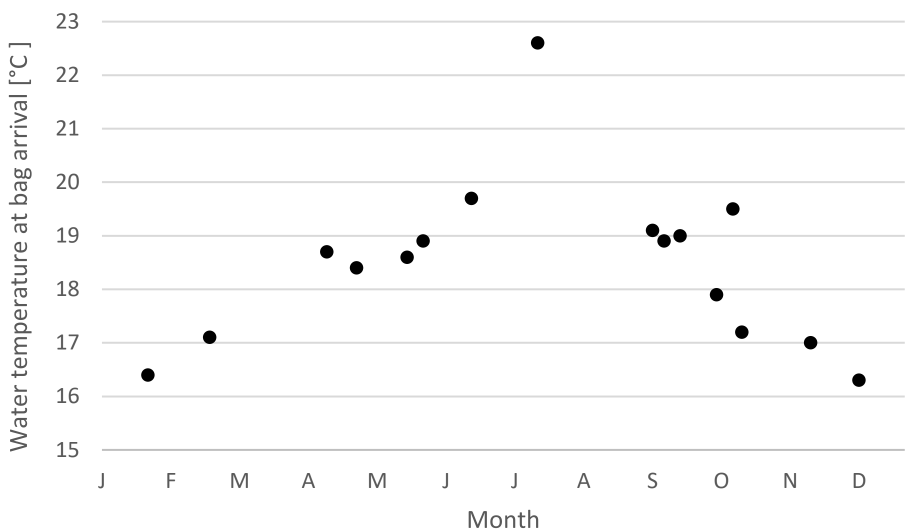

Figure 1 – Seasonal relevance of bag arrival temperatures from post-larvae air shipping (n=16).

<u>Oxygen:</u>
The transport bags are packed with 25-30% conditioned seawater with a PL density of appr. 500-700 per L, filled up with pure oxygen and sealed. Therefore, it is to be expected to have an equilibrium between the gases dissolved in the water and bag atmosphere. However, as pure oxygen is used in the bags and dissolved oxygen measurements are related to air (5 times less oxygen) an oversaturation is measured. Table 1 shows arrival oxygen saturations still exceeding the limit of measurement device (>265%) for the air shipments. A good indication is also if the bags are still under pressure.

If dissolved oxygen during acclimation is lower than 3.0 mg/L, a fine pore diffuser with pure oxygen can be used to gently bubble oxygen into the water. Usually this is not needed during acclimation and should be avoided as much as possible. During oxygenation care must be taken to avoid abrupt pH rises.

An important warning: Never place air stones into bags or acclimations tanks with just arrived PL's. The dissolved carbon dioxide is stripped, causing the pH to abruptly climb. Through this pH rise, an increasing part of the total ammonia nitrogen in the water is converted from the non-toxic ionized form to the toxic un-ionized form (NH_3-N). Therefore, ammonia toxicity can be induced by simply placing an air stone into the water.

TAN / NH_3-N:
Table 1 shows arrival TAN concentrations between 1-9 mg/L and NH_3-N concentrations 0.00 – 0.03 mg/L indicating a high variance between hatcheries. The reason is that some hatcheries are using ammonia removing agents to avoid cumulation. However, due to the low pH the NH_3-N concentrations are usually in a safe range. But care must be taken especially during acclimation from high TAN levels.

Alkalinity and CO_2:
Table 1 shows the arrival conditions for alkalinity ranging from 6.5-9.0 °dH (116-161 mg/L $CaCO_3$) and CO_2 concentration from 17-38 mg/L. Alkalinity is in the expected range for *L. vannamei* production. The elevated CO_2 concentration is also expected but far above the best practice concentration of 6 mg/L. Based on Furtado et. al (2017) concentrations above 23.8 mg/L should be avoided to not induce mortalities. However, it is to expect that due to higher oxygen partial pressure the CO_2 toxicity is reduced. That might explain that also at higher CO_2 concentrations no significant mortality has been observed.

Recommendation for acclimation:

The best way to acclimate post-larvae from plastic bags after long transport is to anticipate the arriving water conditions in the bags. In fish usually the water conditions of the nursery system or quarantine system is set to these parameters and after fish transfer acclimation can start. However, based on our experiences this procedure is gentle but not expedient for post-larvae. The reason is that usually acclimation takes too long. After the long transport time the conditions must be set as quick as possible in a range that post-larvae eat and return to their physiological needs. The method can be described as “Quick but reasonable”. However, the anticipation of arriving water conditions in the bags is essential. By preparation of at least the same volume as the water volume in all the bags, the so called “dilution water” cuts in half the concentration of the most critical parameter NH_3-N by adding it directly to the bags, acclimation tanks, or nursery tanks.

To prepare the dilution water set the following parameters to the anticipated values and adjust based on measured values in the bags upon arrival.

- Temperature can be controlled and set by a heat exchanger or using ice / heater
- pH value can be controlled by HCL (to lower pH) or NaOH (to rise pH)
- Salinity can be controlled by chlorine free tap water (to lower salinity) or a commercial artificial sea salt / natural seawater (to rise salinity). Note: An artificial sea salt solution to increase salinity must be prepared 24 hours before post-larvae arrival. Concentrations up to 150g/L can be prepared. Use pumping to mix up the sea salt. Avoid aeration as it might have negative impacts on the ion composition. Use only commercially available sea salt mixtures for marine aquariums or aquaculture.

Usually dilution water is prepared the day before arrival by setting salinity and pH to the anticipated values. Temperature control is usually done with food grade ice right away before post-larvae transfer. The water parameters before adding it to the post-larvae should be almost the same. Slight differences may be acceptable:

- Temperature ± 1-2 °C
- Salinity ± 1-2 g/L
- pH value - 0.10-0.20, + 0.05-0.10

Afterwards acclimation can start either in special acclimation tanks, that can be set easily to the right size for the arriving post-larvae stage (150-200 post-larvae per liter) or directly in the tanks of the nursery system, which are usually bigger. By setting and increasing the flow into these basins the relevant water parameters are shifted "quick but reasonable" to the target values. The shift is depending mainly on the following parameters (an example is presented in Table 4 and Figure 2): difference between inflow and basin concentration, water inflow rate and basin water volume.

As these values are changing with time they must be controlled and adapted continuously. For example, in the beginning the difference between inflow and basin concentration is the highest and the basin water volume the lowest. Therefore, the water inflow must be set to the lowest value during acclimation to not exceed the recommended acclimation shift for certain water quality parameter.

Please note again that acclimation should be gentle, but the priority is to attain water parameters to enable the post-larvae to eat as soon as possible. Start adding water from your nursery or production system in a slow continuous flow while checking parameters on regularly basis. Increase or decrease flow rate of your water depending on your acclimation table. An example is presented in Table 4 and Figure 2. During acclimation feeding enriched Artemia nauplii may be an option if temperature is above 20°C and post-larvae are active. It is recommended to feed at the same time also a small dose of nursery feed (pellet size 400µm). Keep track on the feed uptake and add feed very carefully if water parameters are good and the PLs are still active. Be careful not to overfeed, as this might decrease water quality.

Procedure for acclimation Förde Garnelen:

Preparation of nursery system:

- Cleaning of the nursery system and/or acclimation tanks following best practices, take care to avoid any sources of H_2S and turbidity, mechanical cleaning is usually sufficient, make sure to rinse properly, when using appropriate chemicals
- Calibration of all probes within the system

- Preparation and calibration of handheld probes for temperature, salinity, pH value and oxygen
- Make sure that nursery system and/or acclimation tanks are working
- Prepare tank outlets with post-larvae size adapted screens (500µm)
- Prepare dilution water to the anticipated bag arrival conditions:
    - Increase salinity using artificial sea salt solution (as already mentioned at least 24 hours before post-larvae stocking)
    - Lower pH value by using HCL dosages. Use hand stirring or pumping to mix it with the water. This you can also prepare the day before, but do not use aeration. Otherwise the pH will rise again.
    - Lower temperature using food grade ice. Use hand stirring or pumping to solve the ice.
- Option: Store min. 100mL sample of dilution water in the fridge until 48 hours after post-larvae arrival for later measurements if needed.

Post-larvae arrival:

- Open cartons carefully, avoid sudden light changes
- Open a relevant number of bags to measure arrival water parameter as claimed in Table 1.
- Check all bags for abnormalities as high mortalities, abnormal behavior, turbid or smelly water
- Store min. 100mL water sample of each of the sampled bags in the fridge until 48 hours post-larvae arrival for later measurements if needed.
- If the difference of mean values over all sampled bags to the dilution water is as the acceptable difference, set the dilution water to the right values
- Fill acclimation tank or nursery tank with dilution water, no water flow
- Transfer 1-2 bags into the prepared water and check swimming behavior for at least 10min before transferring next ones
- Transfer all bags into the prepared basins
- After stocking post-larvae, set water flow to not exceed the acceptable acclimation shift. An example is presented in Table 4 and Figure 2. Set all alarms available.
- Document all relevant water quality parameters at least every 2 hours by hand or data logger
- Take note of any abnormalities during acclimation as increasing mortalities, unusual swimming behavior, physical symptoms and react accordingly as more frequent data sampling, reduction of water flow, etc.
- Prepare acclimation sheet for documentation

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Attachments

Table 2 and Table 3 are presenting an overview about all relevant data to set an acclimation protocol.

Table 4 and Figure 2 are presenting a simulation for the acclimation of US post-larvae in a typical European land-based shrimp farm. It is assumed that acclimation basins are used in a flow-through with water from the production system. Figure 2 is presenting the results from the simulation run.

Table 5 and Figure 3 are presenting the data and simulation for an acclimation of post-larvae from a German hatchery in November 2020.

Table 6 can be used to determine the NH_3-N concentration by pH value and total ammonia concentration. Table 7 can be used to determine the CO_2 concentration by pH and alkalinity.

Table 2 - Standard parameter for post-larvae arrival, transfer, and acclimation procedure

	Temperature	Salinity	pH	Oxygen
	°C	g/L		%
Packing US hatchery	19-23	29-33	7,7-7,9	>>100
Expected arrival at European farm	17-23	29-33	6.7-7.1	>>100
Preconditioning of dilution water	20-22	30-31	7.0-7.1	100
Acceptable difference transfer	±1-2	±1-2	+ 0.05-0.10 - 0.1-0.2	not relevant*
Acceptable acclimation 15 min shift	0.5-1.0	0.5-1.0	0.05-0.1	not relevant*

** but oxygen concentration >3mg/L*

Table 3 – Additional parameter for post-larvae arrival

	Ammonia		Alkalinity		CO_2
	ppm TAN	mg NH_3-N	°dH	mg/L $CaCO_3$	mg/L
Packing US hatchery	<0.1	0.00	7.6-8.4	136-150	2-4
Expected arrival at European farm	1-9	<0.03	6.5-9.0	116-161	17-38
Preconditioning of dilution water	<0.1	0.00	7.6-8.4*	136-150*	14-20

** values correspond to typical artificial sea salt mixtures, lower buffered mixture are an alternative to lower CO_2 value*

Table 4 – Assumed standard parameter for the acclimation simulation Figure 2

Water	Temperature °C	pH	Salinity Ppt	kH °dH	CO2 ppm
Bag water at arrival	20	6.9	31	8.0	24.1
Inflow acclimation basin	30	7.5	15	8.0	5.8

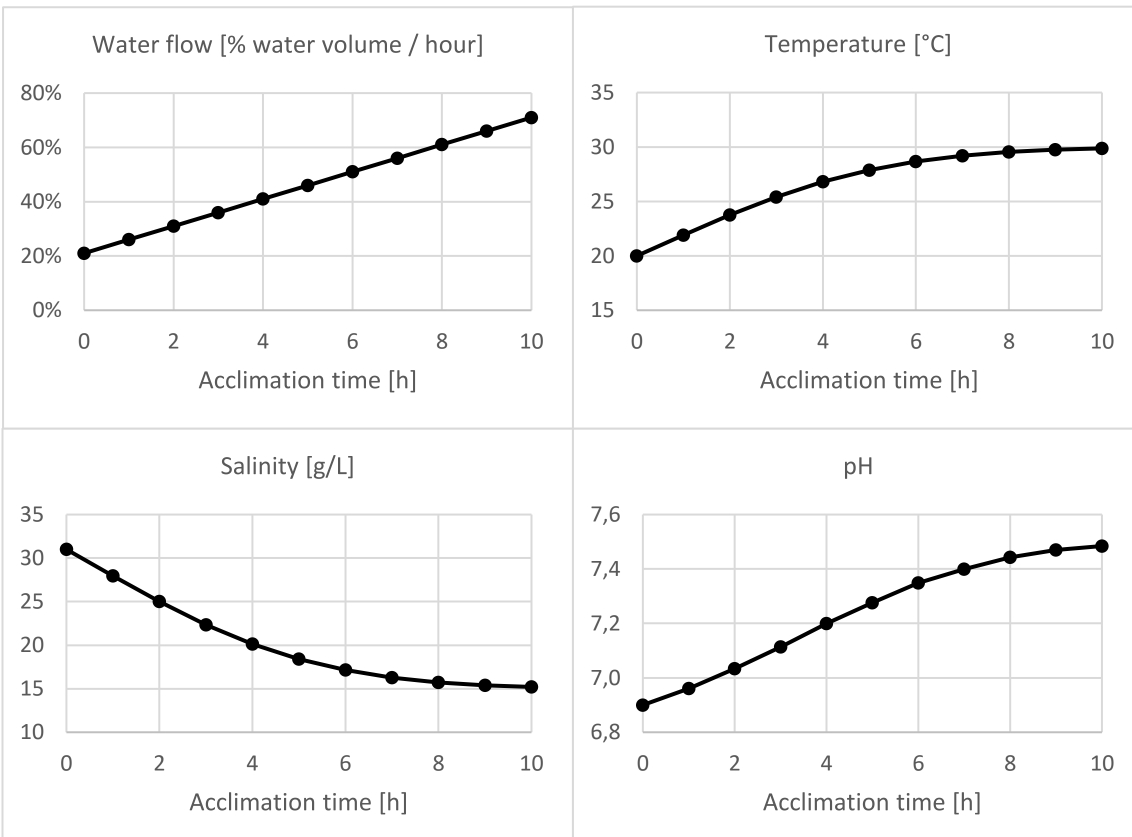

Figure 2 – Simulation of acclimation using the standard parameter in Table 4. The water flow rate was set almost to maximum considering the following restrictions: temperature <3°C/hour, salinity <3g/L/hour and pH <0.3/hour. In this example salinity was the crucial parameter limiting the water flow rate.

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Table 5 - Standard parameter for post-larvae acclimation from Suburban Seafood (November2020) used for simulation in Figure 3

Water	Temperature °C	pH	Salinity Ppt	kH °dH	CO2 ppm
Bag water at arrival	22.0	7.3	27	6,5	8,4
Inflow nursery basin	29.8	8.0	16.6	6,0	1,4

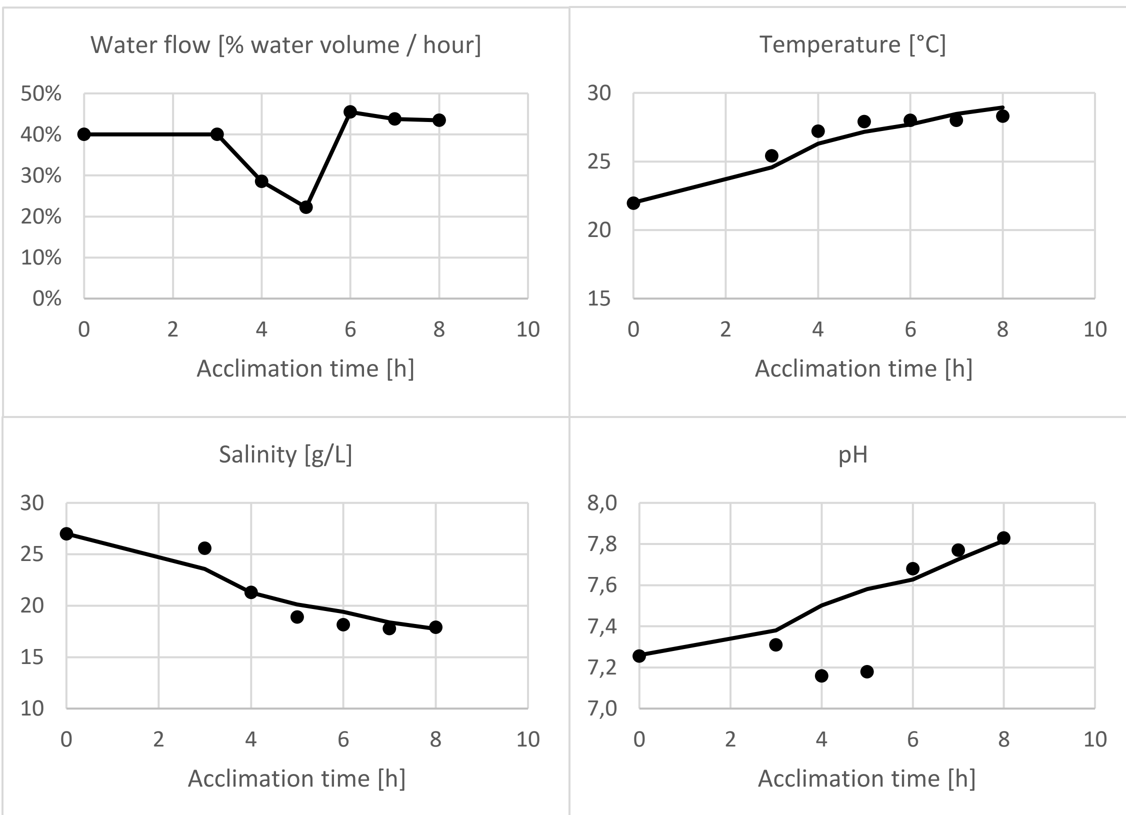

Figure 3 – Acclimation data (dots) of the Suburban Seafood post-larvae delivery in November2020 and simulation of acclimation (line) using the standard parameter in Table 5. The water flow rate of simulation was set to the real data. In this example the maximum measured shifts were: Temperature 1.8°C/hour, Salinity -4.3g/L/hour, pH value -0.15/hour. Differences to simulation and acceptable acclimation shifts, as seen in salinity and pH between hour 3-6, are caused by a strong stratification of the water as the inflowing low salinity water is covering the bag arrival water. Based on experiences this is not negatively affecting the health of the post-larvae but complicates the measurement and interpretation of data especially if using fixed measurement probes and automatic data logging. During this acclimation, no mortalities have been observed.

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Table 6 - Calculation of NH_3-N concentration (FAN) by pH value and total ammonia concentration (TAN) for a temperature of 20°C and salinity of 30g/L. Note: Contrary to the salinity the effect of temperature is urgently to consider. An increase from 20°C to 30°C doubles NH_3-N concentration.

NH_3-N (FAN) µg/L		**pH value**																				
		6.5	**6.6**	**6.7**	**6.8**	**6.9**	**7.0**	**7.1**	**7.2**	**7.3**	**7.4**	**7.5**	**7.6**	**7.7**	**7.8**	**7.9**	**8.0**	**8.1**	**8.2**	**8.3**	**8.4**	**8.5**
Total ammonia concentration (TAN) mg N / L	**0.50**	1	1	1	1	1	2	2	3	3	4	5	6	8	10	13	16	20	25	31	38	47
	1.00	1	1	2	2	3	3	4	5	7	8	10	13	16	20	25	32	40	50	62	76	94
	1.50	2	2	2	3	4	5	6	8	10	12	15	19	24	30	38	48	60	74	92	114	141
	2.00	2	3	3	4	5	7	8	10	13	16	21	26	32	41	51	64	79	99	123	153	188
	2.50	3	3	4	5	7	8	10	13	16	20	26	32	41	51	64	80	99	124	154	191	235
	3.00	3	4	5	6	8	10	12	16	20	25	31	39	49	61	76	95	119	149	185	229	282
	3.50	4	5	6	7	9	11	14	18	23	29	36	45	57	71	89	111	139	173	215	267	329
	4.00	4	5	7	8	10	13	16	21	26	33	41	52	65	81	102	127	159	198	246	305	377
	4.50	5	6	7	9	12	15	19	23	29	37	46	58	73	91	114	143	179	223	277	343	424
	5.00	5	7	8	10	13	16	21	26	33	41	51	65	81	102	127	159	199	248	308	381	471
	5.50	6	7	9	11	14	18	23	28	36	45	57	71	89	112	140	175	219	272	338	419	518
	6.00	6	8	10	12	16	20	25	31	39	49	62	77	97	122	153	191	238	297	369	458	565
	6.50	7	8	11	13	17	21	27	34	42	53	67	84	105	132	165	207	258	322	400	496	612
	7.00	7	9	12	14	18	23	29	36	46	57	72	90	113	142	178	223	278	347	431	534	659
	7.50	8	10	12	16	20	25	31	39	49	61	77	97	122	152	191	239	298	371	462	572	706
	8.00	8	10	13	17	21	26	33	41	52	65	82	103	130	163	204	255	318	396	492	610	753
	8.50	9	11	14	18	22	28	35	44	55	70	87	110	138	173	216	270	338	421	523	648	800
	9.00	9	12	15	19	23	29	37	47	59	74	93	116	146	183	229	286	358	446	554	686	847
	9.50	10	12	16	20	25	31	39	49	62	78	98	123	154	193	242	302	377	470	585	724	894
	10.00	10	13	16	21	26	33	41	52	65	82	103	129	162	203	254	318	397	495	615	763	941

Table 7 – Calculation of CO_2 concentration by pH value and alkalinity (1 °dH = 17.86 mg/L $CaCO_3$) for a temperature of 20° and salinity of 30g/L. Note: The effect of temperature and salinity is not necessarily to consider.

CO_2 (mg/L)		**Alkalinity [°dH]**																			
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
pH value	**6.50**	8	15	23	30	38	46	53	61	68	76	83	91	99	106	114	121	129	137	144	152
	6.60	6	12	18	24	30	36	42	48	54	60	66	72	78	84	90	96	102	108	115	121
	6.70	5	10	14	19	24	29	34	38	43	48	53	57	62	67	72	77	81	86	91	96
	6.80	4	8	11	15	19	23	27	30	34	38	42	46	49	53	57	61	65	68	72	76
	6.90	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
	7.00	2	5	7	10	12	14	17	19	22	24	26	29	31	34	36	38	41	43	46	48
	7.10	2	4	6	8	10	11	13	15	17	19	21	23	25	27	29	30	32	34	36	38
	7.20	2	3	5	6	8	9	11	12	14	15	17	18	20	21	23	24	26	27	29	30
	7.30	1	2	4	5	6	7	8	10	11	12	13	14	16	17	18	19	20	22	23	24
	7.40	1	2	3	4	5	6	7	8	9	10	11	11	12	13	14	15	16	17	18	19
	7.50	1	2	2	3	4	5	5	6	7	8	8	9	10	11	11	12	13	14	14	15
	7.60	1	1	2	2	3	4	4	5	5	6	7	7	8	8	9	10	10	11	11	12
	7.70	0	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10
	7.80	0	1	1	2	2	2	3	3	3	4	4	5	5	5	6	6	6	7	7	8
	7.90	0	1	1	1	2	2	2	2	3	3	3	4	4	4	5	5	5	5	6	6
	8.00	0	0	1	1	1	1	2	2	2	2	3	3	3	3	4	4	4	4	5	5
	8.10	0	0	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	3	4	4
	8.20	0	0	0	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	3	3
	8.30	0	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
	8.40	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
	8.50	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	2

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